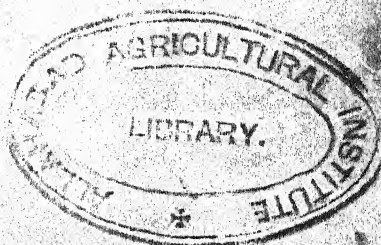


# SOIL SCIENCE

*Founded 1916 by Jacob G. Lipman*

Editor-in-Chief  
FIRMAN E. BEAR

Associate Editor  
HERMINIE BROEDEL KITCHEN



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## LOSSES OF NITROGEN AND ORGANIC MATTER FROM DRY-FARM SOILS<sup>1</sup>

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*Utah Agricultural Experiment Station*

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Loss of nitrogen and organic matter from semiarid cultivated soils is one of the major problems of dry-land agriculture. The original low supply of nitrogen together with the depleting characteristics of alternate wheat and fallow has the possibility of making nitrogen rather than moisture the limiting factor of crop production in certain dry-farm areas. Numerous studies of this problem have been made in humid sections with results almost universally showing losses ranging from 20 to 40 per cent in wheat-growing regions after 20 to 40 years. In arid areas the published data fail to show a similar trend in all cases. Wilsdon and Ali (15, p. 122) in a study of cultivated soils in India under a rainfall of 15 inches or less reported large increases in nitrogen content in certain seasons. They assert that many soils in India, cropped for centuries with little or no addition of manures, still produce good yields in years of sufficient rainfall. Bradley (2) found in eastern Oregon that cropped dry-farm soils as compared to virgin land showed no loss of nitrogen after 25 years of cultivation. Thatcher (14) made similar observations in eastern Washington in areas varying in rainfall from 12 to 23 inches. On the other hand, Stephens (10) has reported appreciable losses of both nitrogen and organic matter from fields in Sherman County, Oregon, as well as from plots at the Moro Branch Station. Likewise, Sievers and Holtz (9) have shown that losses from cropped land as compared to virgin soil in eastern Washington amounted to 22.1 per cent for nitrogen and 34.5 per cent for organic matter.

On the dry-farm lands of Nebraska, Russel (7) found that losses of organic matter varied from 6.5 per cent for fields which had been cropped from 3 to 7 years to 28.0 per cent for lands cultivated from 45 to 60 years. In addition, farms which had suffered from water erosion showed an average loss of 56.0 per cent as compared to 27.1 for level uneroded parts of the same fields. Results from western Kansas procured from the Hays, Colby, and Garden City stations and reported by Gainey, Sewell, and Latshaw (3) indicate that, in rotation tests, losses of organic carbon varied from 8,000 to 14,000 pounds per

<sup>1</sup> Contribution from the department of agronomy and the department of bacteriology and biochemistry, Utah Agricultural Experiment Station. Published with the approval of the director.

<sup>2</sup> Associate agronomist and research professor of agronomy.

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acre, and losses of nitrogen, from 600 to 800 pounds. Under continuous wheat culture, including wheat and fallow, the carbon losses varied from 1,200 to 5,400 pounds per acre at Hays and from 6,000 to 8,000 at Garden City and Colby. In comparing virgin and cropped land Swanson and Latshaw (13) found that losses of nitrogen averaged 32.13 per cent in the humid parts of Kansas, 23.75 per cent in the subhumid, and 20.48 per cent in the semiarid. Reporting results of a survey of some of the dry-farm areas in Utah, Stewart and Hirst (12) found no indication of perceptible nitrogen losses after 10 or more years of cultivation. In another survey of farms in the Cache Valley area of Utah, Stewart (11) reported a gain of nitrogen in the surface soil; the average percentages of nitrogen for these farms follow: first foot—wheat land 0.2055, virgin land 0.1984; second foot—wheat land 0.1466, virgin land 0.1823. In the first foot the wheat land was only 3.45 per cent higher in nitrogen than the virgin soil, but in the second foot the cropped fields were 19.58 per cent lower than the uncropped land.

#### EXPERIMENTAL METHODS

This study was planned to determine the changes in nitrogen and organic matter which have taken place in the cropped dry-farm lands of Utah as compared with virgin soil. In setting up the survey, all fields chosen for sampling were immediately adjacent to virgin unplowed land, which still supported a cover of native vegetation. Such areas as railroad right of ways, old roadways, and good native pasture land were selected. Nine such combinations were found in Cache Valley in northern Utah, and twelve were located in Juab Valley in central Utah. Five samples were taken to a depth of 3 feet at each of five locations in every field, and a like number from the same number of locations on the adjacent virgin area. The locations on the cropped and adjacent virgin areas were never more than 2 or 3 rods apart. The five samples from each location were then mixed, and a composite was taken for analysis. Total nitrogen was determined by the Kjeldahl method, and percentage of organic matter was measured by the rapid Schollenberger test. The organic carbon content of several samples was determined by dry combustion to check the accuracy of the Schollenberger method. Close agreement was found when the total organic matter content was divided by the factor 1.724.

The soils in Cache Valley were laid down in prehistoric Lake Bonneville and have since been worked over by surface agencies. In texture the soils of the fields sampled varied from silt loams to clay loams. The Juab Valley soils have been weathered from adjacent mountain ranges. In texture these soils are usually classed as clay loams. The annual precipitation in Juab Valley averages 13.21 inches, and that in Cache Valley, 16 to 17 inches depending on location.

The cropping system followed on all farms included in the study consisted of alternate wheat and fallow, plowing for fallow being done mostly in early spring followed by sufficient summer tillage to keep weeds under control.



A second part of the study consisted of an attempt to measure some of the factors considered to be responsible for loss of nitrogen and organic matter from soil. Since temperature is known to influence the nitrogen and organic matter content of soil, variations in temperature were measured. In both 1934 and 1935 three thermograph readings were taken, one on fallow land, one on land in crop, and one from a virgin sagebrush area for the summer periods. The temperature bulb was buried 2 inches under the surface in each case.

In addition, a laboratory study was made in which a sample of soil was taken from one of the farms in Cache Valley. The sample, divided into four parts, was maintained at temperatures of 31 and 65°C., with one part for each temperature exposed to ultra-violet light while the other was left untreated. A Hanovia ultra-violet lamp, set at 18 inches above the soil, was used for irradiation.

## RESULTS AND DISCUSSION

### *Nitrogen and organic matter losses*

The data showing comparisons between the nitrogen and organic matter contents of virgin and cropped dry-farm lands in Cache and Juab Valleys are given in tables 1, 2, 3, and 4.

Upon examination of table 1, showing results of the survey of nine farms in Cache Valley, it will be observed that a loss of 15.9 per cent of the nitrogen occurred in the first foot and 14.8 per cent in the second to third foot, as compared to adjacent virgin land. When analyzed statistically both differences were highly significant. A similar comparison of results from Juab Valley, as indicated in table 2, shows that these soils lost 14.5 per cent of the nitrogen in the surface foot and 10.6 per cent in the second to third foot, both differences likewise being calculated as highly significant. A comparison of the data in tables 1 and 2 will show that the nitrogen percentage of Juab Valley soils is only slightly more than half that of Cache Valley soils. Since the mean annual temperature for Cache Valley as taken at Logan is 47.2°F. and that for Juab Valley as recorded at Levan is 47.5°F., it appears that the difference in rainfall is the controlling factor in regard to the difference in nitrogen and organic matter contents of the two areas. According to Jenny (4), where the temperature is constant the nitrogen and organic matter contents of grassland soils increase logarithmically with increasing humidity factors. Though the soils of these two areas are not classed as grassland soils, yet a similar relationship appears to exist.

The data given in the last three columns of tables 1 and 2 represent an attempt to determine the amount of nitrogen taken from the soil by the crops, the total amount lost from the soil, and the amount lost and unaccounted for. The amount of nitrogen removed by crops of wheat, though only an estimate, is considered to be within reasonable limits of accuracy. Each land owner was questioned as to production and protein content of wheat, and in several cases actual records were available. The means for both Cache and Juab

Valleys show that twice as much nitrogen was lost and unaccounted for as was removed by the crop. Sievers and Holtz (9) found a similar situation existing in Washington soils. Their results indicated that 50 per cent more nitrogen

TABLE 1

*Comparison of nitrogen percentages with losses between cropped and adjacent virgin dry-farm land on nine fields in Cache Valley, Utah*

FARM OWNER	NUMBER OF YEARS CROPPED	DEPTH	NITROGEN IN SOILS		LOSS	NITROGEN PER ACRE REMOVED BY CROP	NITROGEN PER ACRE LOST FROM SOIL	NITROGEN PER ACRE UNACCOUNTED FOR
			Virgin	Cropped				
		<i>feet</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
Benson	42	1	.198	.166	16.2			
		2-3	.085	.075	11.8	676	2,080	1,404
Partington	50	1	.179	.145	19.0			
		2-3	.094	.085	9.6	775	2,080	1,305
Green	47	1	.177	.158	10.7			
		2-3	.083	.069	16.9	648	1,880	1,232
Riter	47	1	.175	.152	13.1			
		2-3	.089	.076	14.6	620	1,960	1,340
Farrell	63	1	.215	.172	20.0			
		2-3	.112	.094	16.1	1,082	3,160	2,078
Peterson Bros.	40	1	.164	.145	11.6			
		2-3	.119	.091	23.5	620	3,000	2,380
Myron Hansen	37	1	.240	.210	12.5			
		2-3	.133	.127	4.5	608	1,680	1,072
C. A. Peterson	37	1	.262	.197	24.8			
		2-3	.143	.112	21.7	751	5,080	4,329
J. J. Larson	40	1	.209	.178	14.8			
		2-3	.101	.086	14.8	833	2,440	1,607
Means.....		1	.201	.169	15.9			
		2-3	.107	.091	14.8	735	2,560	1,825

Differences for comparison of virgin { 1 foot.... .012  
and cropped means: 1 per cent point { 2-3 feet.... .009

was removed than could be accounted for. Several other workers have published similar results.

It is admitted that, in making the above assumptions, an error is involved: the sampling was done only to a depth of 3 feet, whereas wheat roots definitely

TABLE 2

*Comparison of nitrogen percentages with losses between cropped and adjacent virgin dry-farm land on twelve fields in Juab Valley, Utah*

FARM OWNER	NUMBER OF YEARS CROPPED	DEPTH	NITROGEN IN SOILS		LOSS	NITROGEN PER ACRE REMOVED BY CROP	NITROGEN PER ACRE LOST FROM SOIL	NITROGEN PER ACRE UNACCOUNTED FOR
			Virgin	Cropped				
		<i>feet</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
Grace Bros.	28	1 2-3	.101 .063	.090 .060	10.9 4.8	440	680	240
C. Paxman	26	1 2-3	.100 .060	.086 .058	14.0 3.3	410	720	310
Bracken Bros.	26	1 2-3	.110 .066	.098 .058	10.9 12.1	409	1,120	711
J. W. Paxman	26	1 2-3	.100 .053	.083 .048	17.0 9.4	409	1,080	671
J. S. Cowan	26	1 2-3	.096 .062	.088 .055	8.3 11.3	411	880	469
R. Sudweeks	28	1 2-3	.120 .080	.094 .059	21.7 26.2	440	2,720	2,280
Wm. Morgan	22	1 2-3	.097 .060	.088 .054	9.3 10.0	299	840	541
S. Linton	24	1 2-3	.088 .054	.080 .054	9.1 ...	382	320	+62
J. Memmott	26	1 2-3	.111 .073	.103 .063	7.2 13.7	450	1,120	670
Paxman-Belliston	29	1 2-3	.113 .075	.081 .058	28.3 22.7	441	2,640	2,199
Serele-Fowkes	25	1 2-3	.131 .063	.104 .059	20.6 6.3	369	1,400	1,031
Tophin-Byland	22	1 2-3	.148 .089	.134 .087	9.5 2.2	363	720	357
Means . . . . .		1 2-3	.110 .066	.094 .059	14.5 10.6	402	1,186	784

Differences for comparison of virgin { 1 foot.... .004  
and cropped means: 1 per cent point { 2-3 feet.... .004



take nitrogen from greater depths than the 3-foot level in dry-farm soils. It is considered, however, that this error may not change the results materially.

The data showing a comparison between the organic matter content of virgin and cropped land for the nine farms in Cache Valley and the twelve in Juab Valley are given in tables 3 and 4. It will be observed that for the Cache Valley area the loss from the surface foot, in comparison with adjacent virgin land, amounted to 20.4 per cent, and that for Juab Valley, 18.8 per cent. Variance analysis of the results showed that the differences between the means were beyond the 1 per cent level of significance in both instances. Comparison of the data for the individual farms as given in tables 1 and 2, with the corresponding farms as listed in tables 3 and 4, indicates that farms which lost comparatively high percentages of nitrogen also suffered the greatest loss of organic matter, though the relationship is not regular in all cases.

If the organic matter means as given in tables 3 and 4 are converted to carbon and divided by the corresponding nitrogen means (tables 1 and 2) the carbon-nitrogen ratio can be calculated. For Cache Valley the carbon-nitrogen ratio for virgin land was found to be 10.74 and that for cropped land 10.17. The ratio for virgin land in Juab Valley was 10.1 and that for cropped land 9.55. This clearly indicates a narrowing of the carbon-nitrogen ratio as losses occur. Similar results have been presented by Sievers and Holtz (9) and Russel (7).

The data reported in tables 1, 2, 3, and 4 were obtained from dry-farms which showed no noticeable indications of erosion. On some of the steeper slopes, and especially on the hilltops in Cache Valley, however, most of the surface soil has been washed away. Results showing this relationship are given in table 5.

The data in table 5 indicate the excessive loss of nitrogen and of organic carbon on land subject to erosion. White clay hilltops, as the data show, lost 58.5 per cent of the nitrogen and 57.8 per cent of the organic matter as compared to level, uneroded cropped land. In a comparison between virgin land and fields which had suffered from erosion Russel (7) has reported a loss of 56 per cent.

In review of the data showing losses of nitrogen and organic matter from dry-farm soils in Utah, the question of nitrogen and organic matter balance or equilibrium presents itself. Will losses continue, or will they cease, following reduction to some lower level? The results reported by Jenny (5) show that the greatest losses occurred in Missouri in the first few years of cropping and that the percentage of loss decreased with time, but even though the curve representing loss flattened out after 60 years of cropping, it was still downward. The data indicate, however, that the nitrogen level does not decline indefinitely but that the end result may be a new equilibrium at a considerably lower level than the original nitrogen content. Results reported by Gainey, Sewell, and Latshaw (3) from Kansas indicate that when the nitrogen content of the soil with which they work falls to approximately 0.10 per cent, the factors

responsible for additions of nitrogen to the soil counterbalance those tending to cause loss, thereby establishing a nitrogen equilibrium near this level. For the soils in Juab Valley, apparently the equilibrium level is below this point.

TABLE 3

*Comparison of organic matter percentages to a depth of 1 foot with losses between cropped and adjacent dry-farm land on nine fields in Cache Valley, Utah*

FARM OWNER	ORGANIC MATTER VIRGIN	ORGANIC MATTER CROPPED	DIFFERENCE BETWEEN VIRGIN AND CROPPED	LOSS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Benson.....	3.33	2.48	0.85	25.5
Partington.....	3.30	2.35	0.95	28.8
Green.....	3.26	2.75	0.51	15.6
Riter.....	3.22	2.64	0.58	18.0
Farrell.....	4.16	3.32	0.84	20.2
Peterson Bros.....	2.80	2.39	0.41	14.6
Myron Hansen.....	4.80	3.96	0.84	17.5
C. A. Peterson.....	4.93	3.75	1.18	23.9
J. J. Larson.....	3.85	3.10	0.75	19.5
Mean.....	3.74	2.97	0.77	20.4
Difference for comparison of virgin and cropped means, 1 per cent point.....				.107

TABLE 4

*Comparison of organic matter percentages to a depth of 1 foot with losses between cropped and adjacent dry-farm land on twelve fields in Juab Valley, Utah*

FARM OWNER	ORGANIC MATTER VIRGIN	ORGANIC MATTER CROPPED	DIFFERENCE BETWEEN VIRGIN AND CROPPED	LOSS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Grace Bros.....	1.92	1.53	0.39	20.3
C. Paxman.....	1.85	1.51	0.34	18.4
Bracken Bros.....	1.76	1.44	0.32	18.2
J. W. Paxman.....	1.80	1.46	0.34	18.9
J. S. Cowan.....	1.76	1.52	0.24	13.6
R. Sudweeks.....	1.79	1.37	0.42	23.4
Wm. Morgan.....	1.69	1.39	0.30	17.7
S. Linton.....	1.54	1.34	0.20	13.0
J. Memmott.....	1.95	1.72	0.23	11.8
Paxman-Belliston.....	1.99	1.36	0.63	31.6
Serele-Fowkes.....	2.30	1.74	0.56	24.3
Tophin-Byland.....	2.61	2.24	0.37	14.2
Means.....	1.91	1.55	0.36	18.8
Difference for comparison of virgin and cropped means, 1 per cent point.....				.093

As shown by the data in table 2 the average nitrogen content of several virgin areas was near 0.10 per cent, yet significant losses occurred from the cultivated fields. Only one field, the S. Linton farm, showed a gain when the amount of nitrogen taken out by the crop was subtracted from the loss from the soil. This may indicate that nitrogen fixation has become active at this particular level, 0.088 per cent. If this is the case then approximately 0.09 per cent may be assumed to be the equilibrium level for the clay loam dry-farm lands of Juab Valley under the alternate crop and fallow system of cultivation. All of the fields sampled in Cache Valley, however, showed definite losses. The Farrell farm, cropped over a period of 63 years from the time of breaking to the time of sampling, showed a reduction from 0.215 per cent nitrogen to 0.172. If the length of the cropping period is considered and the results of Jenny (5) are used as a basis of comparison, it might be assumed that for silt and clay loam soils in Cache Valley the equilibrium level for nitrogen is about 0.17 per cent or slightly lower, depending upon location.

TABLE 5

*Average percentage of nitrogen and of organic carbon found to a depth of 1 foot at three adjacent locations in Cache Valley*

LOCATION	NITROGEN	NITROGEN LOSS AS COMPARED TO LEVEL LAND	CARBON	CARBON LOSS AS COMPARED TO LEVEL LAND
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
White clay hilltop. ....	.097	58.5	1.07	57.8
On side of clay hilltop. ....	.182	22.2	2.09	17.7
Level, uneroded cropped land. ....	.234	....	2.54	....

#### *Factors promoting nitrogen losses*

Results reported by Russell (8, pp. 375-377) indicate that nitrogen lost from soil at Rothamstead can be accounted for by that removed in the crop and that found in drainage water. On the other hand, Sievers and Holtz (9) have shown that under semiarid conditions in Washington, where little or no drainage occurs, 50 per cent more nitrogen was removed than could be accounted for in the crops harvested. Since a similar situation was found to exist under dry-farm conditions in Utah, an attempt was made to measure some of the factors considered responsible for nitrogen losses. The nitrogen loss unaccounted for by the crops removed may have been due to one or all of the following factors: leaching to lower depths beyond the feeding range of the plant; erosion; denitrification; and volatilization through biological and possibly chemical means.

*Leaching.* Under a limited rainfall, such as is characteristic of semiarid lands, little leaching occurs; however, Bracken and Cardon (1) have shown that whenever the rainfall at Nephi, Utah, over a fallow period, including two winters and one summer, exceeds approximately 20 inches, with the excess



occurring largely during the winter and early spring months preceding the crop year, a certain amount of soil moisture moves below the 6-foot level and, in certain seasons, likely below 10 feet. Since the  $\text{NO}_3$  ion is not readily absorbed by the soil complex, it is obvious that soluble nitrogen may move to lower depths with moisture and be lost to the plant. As an example of this, the following data show the distribution of nitric nitrogen to a depth of 10 feet at the beginning of the crop season of 1923, following a fallow period such as that mentioned:

<i>Depth, feet</i>	<i>Nitric nitrogen, p.p.m.</i>
1.....	0.6
2 } .....	1.0
3 } .....	
4 } .....	1.9
5 } .....	
6 } .....	
7 } .....	2.7
8 } .....	
9 } .....	
10.....	5.0

From these data it will be observed that the major part of the available nitrogen was found at depths likely beyond the reach of plant roots and so might be considered lost. This was the only season, however, in which nitric nitrogen was determined along with soil moisture from 1923 to 1933, inclusive, that leaching below the 6-foot level was found to occur to any appreciable amount, but over the cropping period of the fields sampled there may have been similar seasons. Thus leaching of nitrogen might be mentioned as one of the sources of loss, but it is considered of minor importance in these soils.

*Erosion.* Erosion of surface soil may also be considered as one of the sources of loss of nitrogen and organic matter from the fields studied. The excessive losses shown in table 5 were mainly the result of erosion, but no noticeable loss from erosion was evident on the nine fields sampled in Cache Valley or on the twelve fields of Juab Valley, the data for which are shown in tables 1, 2, 3, and 4. It is admitted, however, that a certain loss from erosion unquestionably occurred, but it is assumed that the amount was small and of no major importance as far as an explanation for losses is concerned, since in both areas sampled, an appreciable loss of nitrogen occurred from the 2-3-foot section.

*Denitrification.* Denitrification as a source of loss of nitrogen from these soils appears unlikely, since the quantity of nitrate nitrogen occurring at any given time is small and the amount of organic material is low. Furthermore, these soils are never saturated with moisture, they are generally well aerated, and nitrifying bacteria are found to depths of 8 and even 10 feet. Hence it

is improbable that denitrification is an important factor in the loss of nitrogen from arid soils.

*Temperature.* Since leaching and erosion have likely been responsible for only minor losses of nitrogen and organic matter from these dry-farm lands, it was considered that temperature differences might be one of the major factors contributing to losses. Accordingly, thermograph recordings were made as

TABLE 6

*Mean temperatures for virgin land, land in wheat, clean fallow, and air at Nephi, Utah*

	AIR	VIRGIN LAND (SAGE)	LAND IN WHEAT	CLEAN FALLOW
	°F.	°F.	°F.	°F.
May 29 to October 23, 1933.....	60.5	62.5	64.0	69.0
May 7 to September 28, 1934.....	65.3	68.0	70.8	74.0

TABLE 7

*Temperature of soil in the field, at the surface and at depths of 1 and 2 inches, under various plant covers and at various air temperatures, Nephi, Utah*

CHARACTER OF SURFACE	DEPTH	AIR TEMPERATURES			
		85°F.	90°F.	95°F.	100°F.
Clean fallow	Surface	106	113	126	132
	1 inch	92	100	109	119
	2 inches	88	95	106	109
Stubble*	Surface	102	109	116	127
	1 inch	91	98	106	115
	2 inches	88	93	100	106
Sagebrush	Surface	90	91	96	103
	1 inch	82	87	91	97
	2 inches	80	85	88	92
Western wheatgrass sod	Surface	88	90	93	96
	1 inch	81	84	90	91
	2 inches	79	82	88	89

\* The stubble left after harvesting a yield of 20 bushels per acre, the average production for the land on which temperature readings were made, was approximately 20 inches high.

indicated in table 6. From the data given it will be observed that in 1933 land in wheat and land in clean fallow were 1.5 and 6.5°F. higher, respectively, than virgin land in sage, and in 1934 the respective differences were 2.8 and 6.0°F.

Temperature readings were also made at various depths and on various surfaces as shown in table 7. In taking temperatures at the surface the mercury end of the thermometer was covered with sufficient soil to prevent absorption of heat by the metal. Readings were made only on clear days between

1 and 2 p.m., when the maximum temperature was at or near the temperature recorded.

The data in table 7 indicate a relationship similar to that shown in table 6—that temperatures on clean fallow land were definitely higher than those on virgin land. Likewise, temperatures as recorded in stubble were higher than those on unbroken land. It will also be observed that land covered with a stand of western wheatgrass was lower in temperature than land supporting a growth of sagebrush. Apparently the grass cover served as better insulating material.

Studies by Jenny (4) have shown that for each 10°C. increase in the mean annual temperature the nitrogen content of soil decreases twofold to threefold. The difference in temperature between fallow and virgin land for the summer period in this investigation was approximately 6°F. This, together with the maintenance of a higher soil-moisture content during the fallow period, both of which increase biological as well as possibly chemical activity, may account for the losses of nitrogen and organic matter from Utah dry-farm soils.

*Temperature and ultra-violet light.* Since temperature is known to influence the nitrogen and organic matter content of soil, and since work reported by Mortenson and Duley (6) indicates that ultra-violet light has an effect on the ammonia and nitrate content of the soil, which was assumed, in turn, possibly to have an influence on total nitrogen, a test was set up for the purpose of measuring the effect of these two factors on a sample of dry-farm soil taken in Cache Valley. For the first 6 months of the test, no moisture was added to the soil, which remained essentially air dry, and the analyses showed no change in either total nitrogen or organic matter. Thereafter, the moisture content was maintained at 15 per cent, which is approximately that of the surface foot of soil when in fallow. Each time moisture was added the soil was stirred. The results of the various treatments are given in table 8. These results indicate that all the treatments promoted loss of organic carbon, that from soil exposed to the high temperature of 65°C. being apparently significant. Irradiation seemed to have no effect. Loss of nitrogen was limited to the samples exposed to the higher temperature.

The galvanized iron containers in which the soil was treated showed some corrosion. Because of this, it was considered inadvisable to conduct bacteriological studies. Another similar test was, therefore, set up, the precaution being taken to prevent metal from coming in direct contact with the soil. Total nitrogen and organic carbon; numbers of microorganisms developing on agar plates; ammonifying, nitrifying, and nitrogen-fixing power of the soil were all determined on the variously treated samples.

The effects of ultra-violet light and temperature on the nitrogen and organic carbon of the soil are shown in table 9. In general, the data indicate the same relationships shown in table 8, that is, organic carbon was lost from all samples, regardless of treatment, and loss of nitrogen was limited to those samples subjected to the higher temperature. For some unexplainable reason, how-

ever, the loss of organic carbon in the latter test did not show the regularity exhibited in the former experiment.

As indicated by the data in both tables 8 and 9, no nitrogen was lost in those portions of soil held at 31°C., but there was loss of organic matter. This change was probably caused entirely by bacterial activity. The samples held at 65°C. showed loss of not only organic matter but nitrogen as well. Since this temperature is above the point at which most bacterial activity ceases in the soil, it is likely that chemical rather than biological changes were involved. It is assumed that part of the loss of organic matter and nitrogen occurs in the

TABLE 8

*Effects on organic carbon and nitrogen contents of soil exposed to ultra-violet light and temperature treatments from June 6, 1934 to April 18, 1936*

TREATMENT	ORGANIC CARBON			NITROGEN		
	6/6/34	4/18/36	Loss	6/6/34	4/18/36	Loss
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
31°C. dark.....	3.33	3.09	7.21	.247	.248	0
31°C. irradiated.....	3.33	3.08	7.50	.247	.252	0
65°C. dark.....	3.33	2.43	27.03	.247	.209	15.38
65°C. irradiated.....	3.33	2.43	27.03	.247	.207	16.19

TABLE 9

*Effects on organic carbon and nitrogen contents of soil exposed to ultra-violet light and temperature treatments from December 30, 1936 to May 20, 1938*

TREATMENT	ORGANIC CARBON			NITROGEN		
	12/30/36	5/20/38	Loss	12/30/36	5/20/38	Loss
31°C. dark.....	3.00	2.73	9.00	.221	.220	0
31°C. irradiated.....	3.00	2.55	15.00	.221	.213	0
65°C. dark.....	3.00	2.68	10.66	.221	.197	10.86
65°C. irradiated.....	3.00	2.54	15.33	.221	.202	8.60

field through the activity of microorganisms, yet these results indicate that loss through chemical means may also be possible.

Since number of bacteria is a measure of biological activity of a soil, the microorganisms were counted in the four variously treated samples at the beginning and at the end of the experiment, with the results shown in table 10.

The number of microorganisms in the soil kept at 31°C. in the dark remained approximately constant, but the irradiated portion showed a definite reduction. The part of the sample maintained at a temperature of 65°C. in the dark indicated a slight reduction in numbers, but the combination of irradiation and high temperature markedly lowered the number of organisms.

The effect of the various treatments on the ammonifying, nitrifying, and nitrogen-fixing powers of the sample of dry-farm soils is shown in table 11. From these data it is evident that the ammonification in the soil kept at 31°C.

in the dark for 2 years remained virtually constant. Likewise, there was no change in the portion maintained at 31°C. and irradiated, indicating that although numbers of organisms had decreased, yet when placed under optimum conditions, the surviving bacteria rapidly increased. The soil held at 65°C. in the dark showed a reduction in ammonifying power in somewhat the same proportion as the reduction in numbers of organisms, and though the irradiated portion maintained at 65°C. indicated further reduction in amount of ammonia accumulated, yet the amount was greater than might be expected in view of the marked reduction in number of organisms as shown in table 8.

TABLE 10

*Microorganisms in a variously treated sample of dry-farm soil at the beginning and at the end of the experiment*

Thousands per gram of soil

TREATMENT	DATE ANALYZED		DECREASE
	12/30/36	5/20/38	
31°C. dark .....	3,500	3,000	500
31°C. irradiated.....	4,500	400	4,100
65°C. dark .....	5,000	3,100	1,900
65°C. irradiated.....	4,500	30	4,470

TABLE 11

*Amounts of ammonia, nitrates, and nitrogen fixed in a sample of dry-farm soil variously treated*

Milligrams per gram of soil

TREATMENT	NH <sub>3</sub> PRODUCED IN 4 DAYS		NO <sub>3</sub> FORMED		NITROGEN FIXED	
	12/30/36	5/20/38	12/30/36	5/20/38	12/30/36	5/20/38
31°C. dark.....	101	105	17.48	19.23	12.17	11.67
31°C. irradiated.....	101	103	17.47	19.42	12.42	10.75
65°C. dark.....	101	87	15.13	1.52	12.69	1.39
65°C. irradiated.....	103	57	17.24	0.09	13.17	0.87

If the nitrifying power of the soil, as indicated in table 9, is considered, it is evident that no change occurred between the fresh untreated soil and that maintained at 31°C. regardless of irradiation. At the higher temperature of 65°C., however, marked reduction was evident, with likely no significant difference between the soil held in the dark and that irradiated. The nitrogen-fixing organisms showed approximately the same proportionate change in activity as that exhibited by the nitrifiers.

#### SUMMARY

Nitrogen and organic matter changes were studied on nine dry farms in Cache Valley, northern Utah, and on twelve in Juab Valley, central Utah.

In Cache Valley, virgin land in the first foot was found to be 15.9 per cent higher in nitrogen and 20.4 per cent higher in organic matter than adjacent wheat land. The second to third foot section on virgin land was 14.8 per cent higher in nitrogen than cropped land. For Juab Valley the same comparison showed a nitrogen loss of 14.5 per cent in the first foot and 10.6 per cent in the second to third foot. The loss of organic matter in the surface foot amounted to 18.8 per cent.

On severely eroded areas in Cache Valley, loss of nitrogen and of organic matter amounted to 58.5 and 57.8 per cent, respectively, as compared to level uneroded land in crops.

For farms in Cache Valley it was estimated that crops harvested from the fields sampled removed 735 pounds of nitrogen per acre out of a total of 2,560 pounds lost from the soil, leaving 1,825 pounds, or 71.3 per cent, unaccounted for. Similar comparisons for Juab Valley showed a total loss of 1,186 pounds of nitrogen per acre, of which 402 pounds were removed by the crop, leaving 784 pounds, or 66.1 per cent, unaccounted for.

The nitrogen equilibrium level for Juab Valley soils was considered to be near 0.09 per cent, and that for Cache Valley soil, approximately 0.17 per cent.

In attempting to account for the nitrogen lost from cultivated dry-farm soil through means other than harvested crops, it was considered that slight losses occurred through leaching and erosion. The major part of the loss, however, is assumed to have taken place in some other way not well understood, likely as a result of chemical and biological changes resulting in volatilization of nitrogen in some form. This may be due to higher temperatures and greater amounts of moisture, particularly during the fallow period.

In a laboratory test conducted with samples of dry-farm soil it was found that ultra-violet light had no apparent effect on either the organic matter or the nitrogen content. The portions held at a temperature of 31°C. lost a small amount of organic matter but no measurable amount of nitrogen. At a temperature of 65°C., however, organic matter as well as nitrogen was lost. The loss at this high temperature was considered the result mainly of chemical rather than biological changes.

In respect to numbers of soil organisms, the irradiated portions showed marked reductions over the soil held in the dark, but the activity of the bacteria, as indicated by the amount of ammonia and nitrates formed and the nitrogen fixed, was reduced only by the high temperature of 65°C.

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# THE pH OF SOILS AT LOW MOISTURE CONTENT

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The determination of pH requires the presence of moisture. The relative ease with which determinations are made at comparatively high dilutions has brought into common use ratios varying from one part of soil in one part of water to one part of soil in ten parts of water. In the determination of the maximum pH values of soils containing considerable calcium carbonate or other highly hydrolyzable compounds, high dilutions are of value. Plants, however, are usually not grown at these high dilutions unless such a condition is unavoidable, and in that case growth is ordinarily most unsatisfactory.

## PLANT RELATIONSHIP TO SOIL pH

In the growth of healthy plants (3), the soil moisture content may become excessive on account of retarded drainage, and the period during which the plants are thus subjected determines whether growth shall be adversely affected. In a well-drained soil, whenever irrigation is practiced, the soil that is wetted soon loses sufficient moisture to approach its field capacity. From there on, the growth of the plant is the chief factor in decreasing the soil moisture content. If the soil is cold and drainage is slow because of the high clay content of the soil, the roots and soil minerals may be subjected to prolonged conditions in which the soil is at or above the field capacity. Inadequate aeration of roots and unavailability of soil nutrients because of prolonged periods of high pH are some of the disturbing factors under these conditions. Deficiency diseases, especially those of any of the four heavy metals—iron, manganese, zinc, or copper—may then become obvious in the tops of the plants. The misuse of irrigation water is probably largely responsible for the occurrence of many of the deficiency diseases, particularly that of iron.

The field capacity of a soil for water approximates the values commonly found for the moisture equivalent, and the pH values determined at these moisture percentages are of considerable importance in the growth of the plants. The pH values when the soil moisture is at or above the moisture equivalent often determine whether the plant shall survive or shall be injured by any of the deficiency diseases.

One phase in the growth of the plant represents that carried on with soil moisture conditions ranging from that of the percentage at the moisture equivalent to that at extreme dilution. In an irrigated soil maintained at the lowest value in this range, the soil moisture percentage much of the time

might be considerably above that of the moisture equivalent. In many soils the problems of aeration, drainage, and deficiency diseases would probably be an accompaniment of such practice. It is common to find that trees grown in soils in this moisture range (2) become chlorotic during the wet winter season and turn green again as the soil moisture is depleted by the trees during the spring and early summer.

A second phase in the growth of the plant is necessarily concerned with the range of soil moisture conditions extending from the moisture percentage at the moisture equivalent to the moisture percentage near the hygroscopic values. Within this range, plants find the most favorable conditions for growth. With soils containing considerable hydrolyzable matter, the pH values decrease rapidly as the moisture percentages decrease (4).

#### METHODS

Although Sorensen's definition of pH applies to an aqueous solution and not necessarily to a two- or a three-phase system, the pH meter was used in this study. The equipment consisted of a Beckman pH meter (laboratory model G) with a shielded glass electrode and a sealed calomel electrode, each having a 10-foot cable and supported by a Beckman spring type electrode holder. Distilled water was used directly from the tinned faucet of the tin pipeline in the laboratory. This line connects with a 500-gallon storage tank which is filled daily. In all determinations, the soil samples were vigorously stirred with a heavy glass rod followed by a horn spoon. Pyrex beakers and a tin container ( $2\frac{3}{4}$  inches high x  $4\frac{1}{2}$  inches diameter), formerly used for storing Petri dishes, were used for the thorough mixing of samples.

The determination of pH in solutions or in soils with moisture percentages near or greatly above the moisture equivalent has been made very rapid and easy of manipulation by means of the glass electrode. Compacting the soil by tapping the beaker containing the mixed sample and pressing the glass electrode and its companion calomel electrode into the soil and repeating the process with corresponding readings of pH, is virtually all there is to the determination of pH in soils near the field moisture content (5, 6).

When moisture percentages considerably below the moisture equivalent are used, the simple compacting of the beaker of soil and the pressing in of the electrodes will not suffice in most cases. Experience has shown that under almost any condition in which the glass electrode is found in the soil mass, an equilibrium pH value will be obtained for that particular set of conditions. Hence, it is essential that the two electrodes, without being removed, be pressed deeper into the compacted soil and the surrounding soil be firmly pressed against them. Care is taken in having sufficient soil to protect the sensitive terminal portion of the glass electrode from striking the bottom of the beaker. It is essential that the beaker have a diameter large enough to permit the insertion of the finger tips in the compacting of the soil about the electrodes.

The pH readings are made in duplicate after each renewal of pressure on both electrodes and the accompanying compacting of the soil about them.

At very low moisture percentages, the horn spoon was used in spreading similar films of moisture, however thinly, over each soil particle. After the electrodes are pressed into soil compacted by gently tapping the base of the beaker on a folded towel, the contact of the electrodes with the soil must not be broken. With some soils the final equilibrium was attained just prior to the splitting of the beaker, so great was the pressure used. When the spring clamp that holds the electrodes at a given level was loosened, the fullest steady pressure of the hands was placed upon this electrode-bearing clamp, and the soil was again compacted about the electrodes.

These electrodes are said to withstand at least 35 pounds of pressure. In practice none has ever broken under the final pressure given; the only time an electrode was lost in this way was when a light and powdery chemical was used in insufficient depth and compactness before the insertion of the glass electrode and hence the necessary bumper did not exist between it and the bottom of the beaker. Gentle and patient swaying of the electrodes has always resulted in their ready removal intact from the soil.

Contact of the soil moisture with the sensitive part of the glass electrode should be most intimate in order to obtain representative equilibria and accurate pH values. The fact that the pH meter readings at the start and finish agree with the known pH value of the buffer solution used is no guarantee that pH readings taken in the interim are accurate and representative of the actual pH of the soil being tested. Data in this regard have been obtained by making use of various pure quartz sands and known buffer solutions. It was through such experiments that the contact of the soil moisture film with the glass electrode was found to require greater attention.

#### EXPERIMENTAL

Of prime importance is the manner of determining the pH of soils at moisture percentages comparable to those found in the field. In the preliminary studies in the field, large pits were dug in certain orchards and shelves were cut at various depths along the wall of the pit directly across the irrigation rows.<sup>1</sup> Various methods were tested in dealing with the contact of the electrodes with the soil. As each method was tried it was accompanied by the recording of the pH readings in the shade above ground. Adequate checks or duplicates were run in order to ensure pH recordings typical for the given sets of conditions.

The first method was to hold the electrodes firmly in place in the soil *in situ*. This was accomplished by cutting a fresh surface in turn on each of the hori-

<sup>1</sup> During the first three months in the progress of an extensive survey of the pH of orchard soils in southern California, the valuable assistance of Cecil Compton was made available.

zontal shelves and holding the electrodes firmly in contact with the undisturbed soil surface. The second method was featured by the holding of the electrodes in contact with the soil at the bottom of two holes slightly larger in diameter than the electrodes. These holes were made in the undisturbed shelves by hammering or pushing bolts to a certain depth into the soil. In the third method the soil on a shelf was rapidly chopped with the edge of a trowel. Immediately thereafter the electrodes were firmly pressed into the soil and the soil was compacted with the finger tips about the electrodes to well above the unshielded part of the glass electrode.

The same portion of soil that was tested while in the shelf was next removed with a trowel. The sample of soil was rapidly mixed with a horn spoon in a tinned container and was quickly placed in a Pyrex beaker. The three above described methods were used again, but in a different order, in the pH determinations of the soil in the beaker. The third method described for the pit was used first: the soil in the beaker was compacted by tapping the beaker on a board and after the electrodes were pressed into the soil, the surface soil was compacted with the finger tips to well above the shielded part of the glass electrode. The first described pit method was next used: the soil in the beaker was well compacted by means of the flat edge of a heavy iron bar and firm contact was made by pressing the electrodes against this hard soil surface. Finally, the second described pit method was used: the electrodes were placed in contact with the bottom of bolt holes made in the beaker of soil that previously had been well tamped with an iron bar. The samples were then stored in closed soil containers.

In the evening in the laboratory at Riverside or in a ranch laboratory the pH values of these same soil samples were again determined in the manner described above for beakers of soil. Upon completion of these pH readings, the soil samples were again returned to the numbered soil containers and the moisture percentages were determined in the usual manner by weighing the containers (using a tare) before and after oven-drying at 105°C. for 24 hours. Additional soil samples were obtained from every location tested and were air dried. These were used for the determination of pH at the 1-5 soil-water ratio.

Data were obtained by these procedures from a large number of soils in orchards in southern California. Table 1 presents the results obtained in representative tests. Data such as the moisture percentage and moisture equivalent are omitted from table 1. A study of the table reveals that the contact of the glass electrode with the soil is a matter of the utmost importance in the determination of pH. The table suggests certain storage changes in the pH of fresh samples and especially the possible effects of gaseous changes, but these will not be considered here. Any of the methods tested, very frequently gave closely agreeing results when adjacent samples from a soil shelf were compared, but despite close agreement there was no assurance that the values obtained represented equilibrium values for the desired set of conditions.

TABLE 1  
Effect of contact of glass electrode with soil on pH values of soils at their field moisture content

SOIL TYPE	DEPTH OF SAMPLE feet	(A) SOIL IN PLACE IN ORCHARD PIT				(B) SAME SOIL AS (A) BUT TRANSFERRED TO BEAKER IN ORCHARD PIT				(C) SAME SOIL AS (B); TRANSFERRED FROM SOIL CONTAINER TO BEAKER IN LABORATORY			
		Electrodes in firm contact with undisturbed soil surface	Electrodes in bolt holes made in undisturbed soil surface	Electrodes pressed into loosened soil; soil then compacted about electrodes	Electrodes in firm contact with surface of heavily compacted soil in beaker	Electrodes in bolt holes in heavily compacted soil in beaker	Electrodes pressed into loosened soil; soil then compacted about electrodes	Electrodes in firm contact with surface of heavily compacted soil in beaker	Electrodes in bolt holes in heavily compacted soil in beaker	Electrodes pressed into loosened soil; soil then compacted about electrodes	Electrodes in firm contact with surface of heavily compacted soil in beaker	Electrodes in bolt holes in heavily compacted soil in beaker	Electrodes pressed into loosened soil; soil then compacted about electrodes
Hanford loam at Bryn Mawr (oranges)	0.5	6.00	6.65	6.95	6.73	6.92	6.92	6.68	6.30	6.90	6.30	6.90	6.90
	1.0	6.50	6.40	6.48	7.02	7.06	7.12	6.16	6.46	6.79	6.46	6.79	6.79
	2.0	5.40	5.62	5.85	6.44	6.53	6.90	6.26	6.55	6.97	6.55	6.97	6.97
	3.0	5.30	5.55	6.39	5.50	6.35	6.96	5.70	6.38	6.82	6.38	6.82	6.82
	3.5	4.39	5.90	6.80	6.60	6.83	7.15	5.58	6.30	6.85	6.30	6.85	6.85
Hanford sandy loam on Dufferin Avenue, Riverside (lemons)	0.5	5.55	5.90	6.00	5.76	6.02	6.78	5.28	5.60	6.67	5.60	6.67	6.67
	1.0	4.60	4.90	5.10	5.34	5.22	5.98	5.42	5.82	6.06	5.82	6.06	6.06
	2.0	5.21	5.76	6.44	5.39	6.00	6.80	5.63	5.93	6.75	5.93	6.75	6.75
	3.0	4.93	5.19	6.16	5.20	5.93	6.80	6.18	6.24	6.70	6.24	6.70	6.70
	3.5	5.45	5.91	6.42	5.18	5.60	6.64	5.30	6.00	6.73	6.00	6.73	6.73
Placentia clay loam at Riverside (oranges)	0.5	4.85	5.34	5.70	5.59	5.62	5.91	5.59	6.05	6.10	6.05	6.10	6.10
	1.0	5.38	5.39	5.69	5.09	5.68	5.96	5.02	5.62	5.97	5.62	5.97	5.97
	2.0	5.20	5.34	5.45	4.60	5.36	5.83	4.70	5.50	5.83	5.50	5.83	5.83
	3.0	5.00	5.26	5.62	5.50	5.76	6.10	5.02	5.61	6.00	5.61	6.00	6.00
	3.5	4.54	4.91	6.10	5.03	5.62	6.10	5.78	5.90	6.40	5.90	6.40	6.40
Yolo loam at Corona (oranges)	0.5	4.60	4.80	5.14	4.48	5.21	5.30	5.28	5.81	6.14	5.81	6.14	6.14
	1.0	4.20	4.48	5.12	4.26	5.48	5.10	5.28	5.73	5.82	5.73	5.82	5.82
	2.0	4.83	4.90	5.80	4.50	5.60	5.61	5.12	5.55	5.70	5.55	5.70	5.70
	3.0	5.64	5.42	5.86	5.30	5.62	6.09	5.52	6.00	6.36	6.00	6.36	6.36
	3.5	5.90	6.02	6.33	5.16	5.80	6.62	6.08	6.14	6.70	6.14	6.70	6.70
Yolo loam at Santa Ana (oranges)	0.5	5.26	5.50	6.40	5.32	5.78	6.50	5.58	6.31	6.93	6.31	6.93	6.93
	1.0	5.90	6.22	6.43	5.89	6.36	6.85	5.50	7.00	7.21	7.00	7.21	7.21
	2.0	6.17	6.26	6.51	6.54	6.48	6.96	6.14	6.90	7.16	6.90	7.16	7.16
	3.0	6.05	5.60	6.20	6.85	6.12	6.09	5.70	6.40	6.90	6.40	6.90	6.90
	3.7	4.50	4.97	5.72	6.38	5.88	6.31	5.36	6.30	6.80	6.30	6.80	6.80

Table 1 makes it clear that a full coverage of the unshielded part of the glass electrode accompanied by the most intimate contact of the glass electrode with the soil gave the highest pH readings. In the bolt holes the glass electrodes remained faintly damp much longer than they did in surface contact with the soil shelf without the holes. The lowest pH readings were those obtained by mere surface contact, whereas the intermediate readings were those in which the electrodes were shielded in bolt holes. Thus without this study of the effect of electrode contact on pH, erroneous pH readings may be reported; not that the pH readings are inaccurate in themselves but that the equilibrium they represent is not the equilibrium that it is desired to measure.

The sensitive part of the glass electrode used consists primarily of the hemispherical glass chamber at the extreme end of the electrode. It is essential to ascertain whether accurate pH readings are obtainable when only a part of the sensitive glass is in contact with the moisture of the sample. One of the early tests conducted was to place the electrodes in beakers containing various depths of concentrated buffer solution which previously was used in large quantities to adjust the pH meter readings to 5.0. The final reading at a depth of about 2 mm. was 5.04, whereas that at about 1 mm. was 5.20. When the beaker was nearly emptied and the electrodes were placed against the heavily moistened walls, a value of 5.24 was obtained. When all of the contents of the beaker were shaken out, contact of the electrodes on the moist glass gave a reading of 5.15. Other tests were made in which the sensitive part of the glass electrode was painted with purified paraffin in various designs. When much of the surface area was coated, the pH readings of the buffer solutions were affected, perhaps because of the inability of the liquid to reach some of the uncoated areas, as well as the reduction in the uncoated areas presented to the solution.

#### *Experiments with quartz sands*

Before a large number of pH determinations were to be made in the field, it was advisable to study first the problem of electrode contact. This was carried out by means of pure quartz sands and concentrated buffer solutions. Ottawa sand was found to pass entirely through the 20-mesh sieve and remain on the 40-mesh. Approximate fractionation of Corona quartz sand gave the following percentage separates: 9 per cent remained on the 40-mesh sieve, 71 on the 60-mesh, 5 on the 80-mesh, 11 on the 100-mesh, and 4 was finer than 100-mesh. Pure quartz flour was much too fine to be held by the 100-mesh sieve.

Various aqueous mixtures of quartz sands were prepared and the pH values determined. The sands were not subjected to any purifying process. Since silicic acid is usually considered as alkaline when in aqueous solution and contains silica as do the sands, it was included in these preliminary pH tests. The results (table 2) for silicic acid are considered to be only approximate.

Table 2 shows that the quartz flour was almost entirely free of moisture

before the mixtures were made. The initial pH of the distilled water was 5.70, and hence in every case the sands raised the pH of the distilled water that was added to them. The increase in moisture percentage brought about increased pH values in the case of silicic acid.

In table 2 it is shown that with 25 to 100 per cent of moisture, the pH values were the greater according to the fineness of the sand. Carbonates, silicic acid, and other hydrolyzable compounds were suspected of bringing about much of the change in pH that accompanied the aqueous dilution of the sands.

In an experiment the pH meter was standardized with a concentrated buffer solution of pH 4.10. When the concentrated buffer solution was added to quartz flour in sufficient amount to produce saturation, the pH reading of the

TABLE 2  
*pH values of aqueous mixtures of quartz sands and of silicic acid*

DISTILLED WATER* ADDED TO 100 GM. OF SAND	OTTAWA QUARTZ SAND	CORONA QUARTZ SAND	QUARTZ FLOUR	MOISTURE PERCENTAGE OF QUARTZ FLOUR AT END OF TEST	DISTILLED WATER* ADDED TO 50 GM. OF C.P. SILICIC ACID	C.P. SILICIC ACID
cc.	pH	pH	pH		cc.	pH
5	6.79	7.47	7.41	4.4	2.5	5.88
10	7.05	7.54	7.46	9.5	5.0	6.21
15	7.02	7.56	....	....	10.0	6.50
20	6.95	7.57	....	....	15.0	6.72
25	6.67	7.59	8.24	24.4	25.0	7.60
35	6.99	7.77	8.62	34.3	35.0	8.03
50	7.14	7.75	8.85	....	50.0	8.10
100	6.92	7.78	8.93	....	75.0	8.28
200	6.75	7.19	....	....	....	....
300	6.44	6.87	....	....	....	....
400	6.84	6.46	....	....	....	....
500	6.27	6.60	....	....	....	....
1,000	6.05	6.40	....	....	....	....

\* pH of distilled water = 5.70.

mixture was 4.12. This mixture was then diluted with an equal volume of the dry quartz flour. The pH of the uniform mixture had now changed to 4.26. This mixture in turn was diluted in a similar manner with an equal volume of dry quartz flour. This was accompanied by an increase in the pH to 4.38. The moisture content of the final mixture was 13.6 per cent.

Quartz flour when added to distilled water (pH 5.82), rapidly increased the pH to 6.68. Because of the apparent hydrolysis of certain substances, mixtures of distilled water with sand were found to offer very little information regarding the effect of electrode contact. Therefore concentrated buffer solutions were next substituted for the distilled water in experiments with quartz sands. The pH meter was standardized with the undiluted buffer solutions to read pH 4.10 or 7.00 at the temperature required. The pH meter standardization was very frequently checked during every series of tests.



Table 3 shows some of the variations in the pH readings when a concentrated buffer solution was added in different amounts to quartz sands of several degrees of fineness. Generally the variations from pH 4.10 were less with the increased dilution of the sands and were by far the greatest with quartz flour. With the concentrated buffer of pH 7.00 the quartz flour gave more accurate results than with the buffer of pH 4.10, and the results with the quartz flour were more accurate with the increased dilution of the flour with the concentrated buffer solution.

TABLE 3

*pH readings obtained when concentrated buffer solutions were added to quartz sands*

CONCENTRATED BUFFER SOLUTION ADDED TO 100 CM. OF SAND	OTTAWA QUARTZ SAND	CORONA QUARTZ SAND	QUARTZ FLOUR
cc.	pH	pH	pH
Buffer solution of pH 4.10			
2.5	4.30	4.30	4.81
5.0	4.28	4.26	4.58
7.5	4.33	4.21	4.58
10.0	4.25	4.17	4.53
15.0	4.21	4.16	4.41
20.0	4.22	4.17	4.36
30.0	....	....	....
35.0	....	....	4.24
Buffer solution of pH 7.00			
2.5	6.91	6.78	6.64
5.0	6.80	6.84	6.78
7.5	6.66	6.86	6.84
10.0	6.64	6.88	6.95
15.0	6.72	6.91	6.96
20.0	6.98	6.88	6.94
30.0	....	....	6.99

The disturbing factors presented in table 3 were sought out by purifying the various sands. The sands were placed in 20-liter wide-mouth glass bottles and were shaken with freshly distilled water. The supernatant solution was removed by decantation after the sand had settled. This procedure was repeated 20 or more times for each lot of sand. The sands were then oven-dried in glass dishes at a low temperature. Some of the determinations reported in table 2 and all of those reported in table 3 were repeated with these purified sands. The quartz flour used contained about 0.05 per cent of moisture. The moisture equivalents (as determined in duplicate) of these purified sands were: Ottawa sand, 0-0.08 per cent; Corona sand, 1.2 per cent; and quartz flour 12.2 per cent.

Table 4 (when compared with table 2) shows that increased dilution of the

purified quartz flour with distilled water increased the pH of the mixture but not so much as before the quartz flour was purified. Purification of the quartz sand by acid extraction before working with distilled water was not attempted.

The purified sands were then used as in table 3. The resulting pH readings are given in table 5. It is apparent that when sufficient buffer is present the pH values at low moisture contents may be determined very accurately when certain factors are given attention. When dealing with low moisture contents, each part of the glass and of the calomel electrodes is best wiped dry with a sheet of tissue paper and then rewiped with a new sheet of the paper. When dealing with high moisture contents such a precaution is not essential. The use of a horn spoon or some agent for thoroughly distributing the surface films and for making a thoroughly uniform mixture was most necessary.

While the pH readings reported in table 5 were being made, it was found that equilibria which may not be the desired ones may be obtained. This may be seen most clearly in the pH readings obtained for the first lot of quartz

TABLE 4

*Effect of small amounts of distilled water on the pH readings of purified quartz flour*

DISTILLED WATER* ADDED TO 100 GM. OF QUARTZ FLOUR	pH READING OF QUARTZ FLOUR- WATER MIXTURE	MOISTURE PERCENTAGE OF QUARTZ FLOUR AT END OF TEST
cc.		
2.5	6.27	2.5
5.0	6.57	4.6
7.5	6.57	7.1
10.0	6.68	9.4
12.5	6.89	12.0

\* pH of distilled water = 5.53.

flour samples as compared with results obtained for the second lot of samples. By the use of pressure in obtaining good contact between the electrodes and the aqueous phase, fairly accurate readings were obtained, provided the contact was repeatedly tested for improvement. The pH values given in table 6 show the effects of the electrode contact with the films of moisture in the sample. The pH readings were first made at the lower temperature and then at a temperature 5°C. higher in order to obtain the pH at the desired temperature by interpolation. The pH determinations reported subsequently to table 6 were conducted with a new pH meter with temperature calibrations at 1° instead of 5°C.

*Experiments with calcium carbonate, calcium sulfate, and sodium chloride*

When the precautions mentioned are observed, pH readings may be obtained at very low moisture contents. With the sample the pH of which is determined immediately after standardization of the pH meter, greater patience may be required before final equilibrium is reached than in dealing with

the other replicate samples that follow in turn thereafter. In the procedure followed 50 or 100 gm. of the powdered chemical was intimately mixed with a definite volume of distilled water by means of a horn spoon in a tinned con-

TABLE 5

*pH readings obtained when concentrated buffer solutions were added to purified quartz sands*

CONCENTRATED BUFFER SOLUTION ADDED TO 100 GM. OF SAND	OTTAWA QUARTZ SAND	CORONA QUARTZ SAND		QUARTZ FLOUR				OTTAWA QUARTZ SAND	CORONA QUARTZ SAND	QUARTZ FLOUR*
		Original- ly deter- mined	Rede- ter- mined after 6 hours	Original- ly deter- mined	Rede- ter- mined after 24 hours	Second lot sam- ples*	Addi- tional sam- ples†			
Buffer solution of pH 4.10								Buffer solution of pH 7.00		
cc.	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
2.5	4.17	4.10	4.16	4.40	4.60	4.10	4.13 4.08 4.09 4.10	6.88	6.99	6.96
5.0	4.11	4.08	4.13	4.35	4.48	4.10		6.90	7.00	6.95
7.5	4.10	4.07	4.09	4.31	4.41	4.12	...	6.93	7.00	7.03
10.0	....	4.08	4.09	4.29	4.35	4.07	...	7.00	6.96	6.99
12.5	....	4.10	4.05	4.24	4.42	4.13	...	6.91	7.00	7.14
15.0	....	4.10	4.08	4.29	4.29	4.14	...	6.93	6.92	6.95
17.5	....	....	....	....	....	4.15	...	7.07	7.00	7.04
20.0	....	....	....	....	....	4.16	...	7.00	6.96	7.00
22.5	....	....	....	....	....	4.13	...	6.97	6.90	7.00
25.0	....	....	....	....	....	4.15	...	6.98	6.98	7.07
Moisture percentage of sand at end of test										
2.5	....	....	2.3	....	....	2.1	2.1 2.2 4.7 4.6	....	....	1.8
5.0	....	....	4.7	....	....	4.2		....	....	4.2
7.5	....	....	7.0	....	....	6.6	....	....	....	6.4
10.0	....	....	9.4	....	....	9.2	....	....	....	8.9
12.5	....	....	11.5	....	....	11.4	....	....	....	11.3
15.0	....	....	14.1	....	....	13.9	....	....	....	13.6
17.5	....	....	....	....	....	16.4	....	....	....	15.9
20.0	....	....	....	....	....	18.7	....	....	....	18.6
22.5	....	....	....	....	....	21.3	....	....	....	20.9
25.0	....	....	....	....	....	23.7	....	....	....	23.3

\* Prepared samples kept overnight in covered beakers; standardizing buffer solution treated similarly.

† pH readings obtained immediately after samples were prepared.

tainer, and the mixture was then transferred to a Pyrex beaker of a size suitable for compacting the material, by means of the finger tips, about the electrodes.

The electrode contact with the moisture films in the samples was renewed by pressing the electrodes deeper into the sample, followed by additional

compacting of the soil about the electrodes. The final pH reading was obtained whenever the pH readings that followed each of three successive electrode contact renewals were virtually unchanged.

TABLE 6  
*Final pH readings as affected by electrode contact*

SIMPLE INSERTION OF ELECTRODES FOLLOWED BY SINGLE COMPACTING OF SAMPLE ABOUT ELECTRODES			REPEATED RENEWAL OF PRESSURE (ELECTRODE CON- TACT) WITHOUT REMOVAL OF ELECTRODES		
2.5 cc. concentrated buffer solution of pH 7.00 added to 100 gm. of Ottawa sand					
20°C.	25°C.	23°C. (temperature of the mixture)	20°C.	25°C.	24°C. (temperature of the mixture)
6.80	6.71	6.75	6.71*	6.83	6.88
6.80	6.71		6.75*	6.84	
6.81	6.71		6.80*	6.85	
6.81			6.90*	6.85	
6.81			6.93*		
			6.97*		
			6.98†		
5 cc. concentrated buffer solution of pH 7.00 added to 100 gm. of Ottawa sand					
20°C.	25°C.	23°C. (temperature of the mixture)	20°C.	25°C.	24°C. (temperature of the mixture)
6.78	6.72	6.75	6.78*	6.88	6.90
6.81	6.73		6.80*	6.88	
6.81	6.73		6.85*	6.88	
6.81			6.91*		
			6.97*		
			7.00*		
			7.00*		
			7.00*		
5 cc. concentrated buffer solution of pH 7.00 added to 100 gm. of Corona sand					
			25°C.	30°C.	26°C. (temperature of the mixture)
			6.78*	6.84	7.00
			6.86*	6.84	
			6.92*		
			7.04*		
			7.04*		
			7.04*		

\* At least two successive similar pH readings were obtained, after which the pressure on the electrodes was renewed and the sample was further compacted about the electrodes.

† Further pressure on the glass electrode in such coarse sand is likely to damage the electrode.

The calcium carbonate powder was from lot 112,937 (Bakers' Analyzed c.p. powder) with percentage impurities listed as follows: SO<sub>4</sub>, 0.09; Cl, 0; heavy metals (as Pb), 0.001; alk. 0.40. This chemical was shaken with 5 gallons of

distilled water, which was poured off after the powder settled. After many repetitions of this procedure, the powder was dried in shallow glass dishes at a low temperature. The moisture equivalent of the purified powder was 23.3 per cent.

TABLE 7  
*pH readings obtained when distilled water was added to calcium carbonate powder*

DISTILLED WATER ADDED TO 100 GM. OF POWDER	C.P. CALCIUM CARBONATE		PURIFIED CALCIUM CARBONATE	
	pH	Moisture percentage at end of test	pH	Moisture percentage at end of test
cc.				
1.0	....	....	{ 8.70* 8.54 8.82*	0.6
2.0	....	....	{ 9.00 8.90	1.3
2.5	9.10	1.7	....	....
3.0	9.10	2.4	....	....
4.0	9.32	3.3	{ 9.09* 8.99	3.6
5.0	9.32	4.2	....	....
6.0	9.30	5.2	{ 9.20* 9.02	5.3
6.3	....	....	9.08	5.7
7.0	9.36	6.2	....	....
8.0	9.41	7.1	9.08	7.3
9.0	9.35	8.0	....	....
10.0	9.31	9.0	9.05	9.4
12.5	....	....	{ 8.90 8.94	{ 11.8 11.9
15.0	9.35	14.1	....	....
25.0	....	....	{ 8.69 8.70	{ 24.0 24.4
50.0	....	....	9.30	48.6
75.0	....	....	9.50	73.6
100.0	....	....	{ 9.34 9.58 9.50	{ .... .... 98.6
500.0	....	....	{ 9.47 9.71	{ .... ....
1,000.0	....	....	{ 9.65 9.70	{ .... ....

\* Replicate use of the same sample.

The samples of powder and distilled water were thoroughly mixed with a horn spoon, and all of the other precautions mentioned were taken. In some cases the same sample was used a second time in order to note the degree of consistency of the results. Table 7 shows the pH changes corresponding to

the various dilutions. With the low moisture percentages the changes in the pH readings were relatively small.

Similar determinations were made with Bakers' Analyzed c.p. calcium sulfate powder ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) of lot number 8135, the label of which indicated the following percentage impurities: Cl, 0.002;  $\text{CO}_3$ , 0.20; Mg and alk., 0.10; Fe, 0.001; N, 0.02; heavy metals (as Pb), 0.002. Table 8 gives the data obtained. The moisture percentages indicate the large amounts of moisture present in such powder and the relative uniformity of the pH readings at all except the highest dilution. Tables 7 and 8 show that even with the smallest additions of distilled water, both compounds gave alkaline pH readings. With

TABLE 8  
*pH readings obtained when distilled water was added to calcium sulfate or to sodium chloride powder*

DISTILLED WATER* ADDED TO 50 GM. OF C.P. CALCIUM SULFATE POWDER	C.P. CALCIUM SULFATE		CALCIUM SULFATE FROM SECOND BOTTLE OF SAME LOT NUMBER		DISTILLED WATER† ADDED TO 100 GM. OF C.P. SODIUM CHLORIDE POWDER	C.P. SODIUM CHLORIDE	
	pH	Moisture percentage at end of test	pH	Moisture percentage at end of test		pH	Moisture percentage at end of test
cc.					cc.		
1.5	7.77	24.6	....	....	..	....	...
2.0	7.69	23.6	....	....	..	....	...
3.0	7.81	24.5	....	....	..	....	...
4.0	7.80	27.7	7.89	27.2	4	5.95	3.9
5.0	7.80	26.8	....	....	..	....	...
6.0	7.88	32.2	7.96	31.7	6	5.97	5.7
7.0	7.79	38.4	....	....	..	....	...
8.0	7.80	40.5	7.83	36.4	8	6.12	6.6
9.0	7.81	41.5	....	....	..	....	...
10.0	....	....	7.91	40.9	10	6.20	8.6
500.0	....	....	8.15	....	..	....	...

\* pH of the distilled water 5.80.

† pH of the distilled water 5.63.

such compounds, the values at the highest dilution varied considerably, depending on the source of the product. This agrees with the experience of Buehrer and Williams (1).

In the experiment with sodium chloride, a c.p. product was purified by two successive recrystallizations, and the resulting chemical was powdered after being dried. Table 8 shows the pH readings obtained when low percentages of moisture were used. Increased dilution slightly increased the pH readings. All of the mixtures tested were acid.

#### *pH of soil at field moisture content*

The importance of the electrode contact may be well illustrated with a soil sample obtained in a closed container on July 8, 1940, from a walnut orchard

near Santa Barbara, California. The sample represented the depth between 3 and 4 feet. The soil was passed through hardware cloth (8 square meshes per inch) in order to remove coarse gritty material and to break up the lumps. A suitable sample of the screened soil was thoroughly mixed with a horn spoon and was placed in a 250-cc. Pyrex beaker, the base of which was tapped several times against a cloth on the table top, in order to compact the soil. The electrodes were then inserted with considerable pressure, and a small additional amount of the soil sample was added, after which the soil was well compacted against the electrodes, care being taken to cover fully the unshielded part of the glass electrode. A new Beckman pH meter was used with temperature corrections for any desired soil temperature. The actual pH recordings were: (27° mixture) 6.56, 6.56, 6.58, 6.60, 6.60, 6.60, 6.61, 6.61, 6.62, 6.63, 6.63, 6.64, 6.64, 6.66, 6.66, 6.67, 6.67, 6.67, 6.67, 6.68, 6.69 (8 times), 6.70 (9 times), 6.72 (18 times). Then the method<sup>2</sup> of reapplying pressure on the electrodes and recompacting the soil about them was used in continuing with this same soil sample. The pH readings were: 6.93\*, 6.97\*, 7.00\*, 7.00\*, 7.00\*, 7.00\*, 7.00\*. The electrodes were removed from the soil, which was then broken up with the spatula and, after being remixed, was returned to the beaker. The pH determinations were repeated as before. The electrodes, after being cleaned and wiped dry, were pressed only once into the soil, and the soil was compacted only once about the electrodes. The pH recordings were: (28° mixture) 6.69, 6.69, 6.70 (4 times), 6.71 (9 times). Then the pressure and recompacting method was used in continuing with the same soil sample, and the following pH readings were observed: 6.89\*, 6.92\*, 6.95\*, 6.97\*, 6.98\*, 6.99\*, 6.99\*, 6.99\*, 6.99\*. The standardization of the pH meter changed inappreciably throughout these and all other tests made in this report.

#### *pH of soils at very low moisture percentages*

Some preliminary pH readings were made on air-dry soil that had been screened through hardware cloth (8 square meshes to the inch). In some cases a second large sample was also screened from the same original field sample, a day or more after the first large sample was screened. No particular care was taken to screen these samples to finer proportions, but considerable care was taken to mix the soil thoroughly in order to distribute the soil films uniformly. Table 9 illustrates the kind of results obtained when the soils contain relatively low moisture percentages.

It is of much interest that at these moisture contents none of the soil mixtures show alkalinity even though at high moisture content some of them react strongly alkaline (4).

<sup>2</sup> The asterisk indicates that at least two successive similar pH readings were obtained, after which the pressure on the electrodes was renewed and the sample was further compacted about the electrodes.

*pH of soils at very widely different moisture percentages*

A large number of soil samples were obtained from representative citrus, walnut, and avocado orchards throughout southern California. Air-dried samples were selected from among these and were passed through a 2-mm. (circular hole) mesh sieve. Determinations of pH, by the method described, were made on these soils to which various amounts of distilled water were added.

Table 10 shows the type of results obtainable when very low as well as very high percentages of soil moisture are employed. Opportunity is also given to compare these results with those obtained when the measurements were

TABLE 9

*pH values of soils at very low moisture percentages*

2.5 cc. distilled water added to 100 gm. of air-dry soil

	RAMONA SOIL TYPE NEAR CITRUS EXP. STATION AT RIVERSIDE		VIRGIN SOIL NEAR FALL- BROOK (2-3- FOOT DEPTH)		YOLO SOIL FROM UNIV. OF CALIF. AT LOS ANGELES		TRAVER SOIL FROM UNIV. OF CALIF. AT LOS ANGELES		HANFORD SOIL (PAS- TURE) NEAR CITRUS EXP. STATION AT RIVERSIDE		ALTAMONT SOIL (OVER- LYING LIME- STONE) NEAR COVINA	
	pH	Mois- ture per- cent- age	pH	Mois- ture per- cent- age	pH	Mois- ture per- cent- age	pH	Mois- ture per- cent- age	pH	Mois- ture per- cent- age	pH	Mois- ture per- cent- age
First samples	6.58	2.7	5.15	4.6	5.50	5.5	6.79	4.0	6.34	3.6	5.19	8.9
	6.62	2.7	5.18	4.6	5.60	5.6	6.80	3.8	6.47	2.6	5.33	8.7
	6.50	2.7	.....	.....	5.63	5.6	7.00	3.7	6.45	2.6	.....	.....
Second samples	.....	.....	5.33	4.4	5.60	5.5	6.92	3.8	.....	.....	5.00	8.5
	.....	.....	5.33	4.4	5.60	5.6	6.93	3.9	.....	.....	5.23	9.0
	.....	.....	5.52	4.4	.....	.....	6.90	3.9	.....	.....	5.40	9.4
Moisture equiv- alent	.....	11.9	.....	16.7	.....	27.4	.....	18.3	.....	8.8	.....	40.2

made in the field. Fairly concordant results were obtained when separate 100-gm. samples of the screened air-dried soil were used at very low moisture percentages, as shown in table 10. In this table, as well as in succeeding tables, the pH values of the first one or two samples at very low moisture content may deviate the most from the mode of the readings. The surprising fact is the very active effect of moisture in the low range of moisture percentages. One of the soils has a very high moisture equivalent—approximately four times that of the other two soils—and as the data show, it also has a high percentage of hygroscopic moisture. From an examination of tables 10 to 13 inclusive, it appears that the pH value of a soil has very little significance unless at the very least it is accompanied by the moisture percentage values.



Table 11 shows the results obtained with soils of low moisture content from avocado orchards. These soils showed a considerable degree of acidity when

TABLE 10

*Effect of moisture content on pH values of soils from citrus orchards*

DISTILLED WATER, CC., ADDED TO 100 GM. OF AIR- DRIED SOIL	VALENCIA ORANGE ORCHARD; 29 YEARS OLD; BARSDALE; IN VENTURA COUNTY; YOLO FINE SANDY LOAM		OLD LEMON ORCHARD; ALTA LOMA IN SAN BERNARDINO COUNTY; HANFORD SANDY LOAM		WASHINGTON NAVEL ORANGE ORCHARD; 25 YEARS OLD; WOODLAKE IN TULARE COUNTY; HEAVY SOIL	
	pH	Moisture percentage	pH	Moisture percentage	pH	Moisture percentage
	Sample 1-2 feet; moisture equivalent 14.2		Sample 0-0.5 foot; moisture equivalent 10.2		Sample 0-1 foot; moisture equivalent 39.9	
0	4.22	1.7	4.62	1.0	4.00	6.9
0	4.32	1.9	4.51	1.0	4.43	7.0
0	4.62	2.0	4.80	1.1	4.40	7.2
0	4.32	1.9	4.66	1.1	4.30	7.3
0	4.47	1.9	....	....	4.20	7.3
0	4.72	1.7	....	....	4.35	7.5
0	4.70	1.7	....	....	4.30	7.4
0	4.69	1.8	....	....	....	....
0.5	....	....	....	....	4.60	8.3
1.0	....	....	5.62	2.1	4.70	8.6
1.0	....	....	5.39	2.1	....	....
1.0	....	....	5.57	2.1	....	....
1.0	....	....	5.53	2.0	....	....
1.0	....	....	5.42	2.2	....	....
1.0	....	....	5.66	2.1	....	....
1.0	....	....	5.52	2.0	....	....
1.0	....	....	5.50	2.1	....	....
1.5	....	....	....	....	5.00	8.9
2.0	....	....	....	....	5.22	9.5
2.5	....	....	....	....	5.28	9.8
Samples at various depths						
500*	7.21	....	6.54	....	7.98	....
500	6.98	....	6.18	....	8.19	....
500	6.90	....	6.82	....	8.42	....
500	7.01	....	....	....	....	....
500**	7.09	....	....	....	8.73	....
Field*	7.19	12.4	6.30	11.7	5.70	24.3
Field	6.65	11.0	5.60	9.4	5.97	26.6
Field	6.70	11.5	6.35	16.2	6.08	27.4
Field	6.73	9.5	....	....	....	....
Field**	6.76	11.0	....	....	5.85	24.5

\*-\*\* Depth (feet): 0-0.5, 0.5-1, 1-2, 2-3, and 3-4, respectively.

field determinations of pH were made. The growth of the trees in every case was of excellent quality. Low sprinklers were used in the irrigation of each of these orchards.

The soils from the orchards reported in table 11 had moisture equivalent values of 7.0, 12.0, and 19.4 per cent, respectively. With soils of this charac-

TABLE 11  
*Effect of moisture content on pH values of soils from avocado orchards*

DISTILLED WATER, CC., ADDED TO 100 GM. OF AIR- DRIED SOIL	FUERTE AVOCADO ORCHARD, 12 YEARS OLD, MT. HELIX AREA IN SAN DIEGO COUNTY; 8 LBS. N, 2 LBS. P, 2 LBS. K; YIELD AVERAGE: 160 LBS. PER TREE		TOP-WORKED SEEDLING AVOCADO ORCHARD, 26 YEARS OLD, ESCON- DIDO IN SAN DIEGO COUNTY; NO ORGANICS OR CULTIVATION; 3 LBS. N PER TREE		LARGE AVOCADO TREES; LA HABRA IN ORANGE COUNTY; RECENT ALLUVIAL BLACK LOAM IN TOP FOOT, GRADING TO FINE SANDY LOAM IN FOURTH FOOT			
	pH	Moisture percentage	pH	Moisture percentage	pH	Moisture percentage		
	Sample 1-2 feet; moisture equivalent 7.0		Sample 0-1 foot; moisture equivalent 12.0		Sample 0-0.5 foot; moisture equivalent 19.4			
0	....	....	....	....	4.00	2.0		
0	....	....	....	....	4.21	2.0		
0	....	....	....	....	4.04	1.9		
0	....	....	....	....	4.20	2.0		
0	....	....	....	....	4.19	2.1		
.5	....	....	....	....	....	....		
.5	....	....	....	....	....	....		
.5	....	....	....	....	....	....		
1.0	....	....	....	....	5.10	3.2		
1.5	5.93	2.2	....	....	....	....		
1.5	5.80	2.2	....	....	....	....		
1.5	5.90	2.1	....	....	....	....		
1.5	6.00	2.2	....	....	....	....		
2.5	6.20	3.1	6.39	3.3	....	....		
2.5	....	....	6.30	3.5	....	....		
2.5	....	....	6.28	3.4	....	....		
2.5	....	....	6.42	3.4	....	....		
10.0	....	....	7.00	10.7	....	....		
	Samples at various depths							
			dry area	wet area	dry area	wet area		
500*	5.27	....	6.90	6.74	...	....	7.40	....
500	5.42	....	7.20	6.99	...	....	7.40	....
500	5.71	....	7.50	7.33	...	....	6.94	....
500	6.35	....	7.61	7.43	...	....	7.03	....
500**	6.67	....	7.68	7.57	...	....	6.94	....
Field*	4.65	10.4	5.41	6.21	8.9	14.6	6.20	19.1
Field	4.98	7.8	5.91	6.15	6.3	12.2	6.25	17.8
Field	5.19	8.6	5.95	6.39	6.2	11.2	5.98	15.6
Field	5.57	8.6	5.97	6.58	6.5	10.6	5.75	12.8
Field**	5.99	8.9	6.05	6.57	6.5	9.8	5.66	10.4

\*-\*\* Depth (feet): 0-0.5, 0.5-1, 1-2, 2-3, and 3-4, respectively.

ter, fairly accurate pH determinations were obtained at extremely low moisture contents. In the dry area samples of the soil from the Escondido district the pH values obtained at the 1:5 soil-water ratio were higher than the cor-

TABLE 12

*Effect of moisture content on pH values of soils from orange orchards*

DISTILLED WATER, CC., ADDED TO 100 GM. OF AIR-DRIED SOIL	VALENCIA ORANGE ORCHARDS						WASHINGTON NAVAL ORANGE ORCHARD	
	10 years old; chlorosis; Placentia in Orange County		12 years old; Rancho Santa Fe in San Diego County; Altamont clay soil		16 years old; sour stock; San Fernando in Los Angeles County; clay loam soil		42 years old; La Verne Heights, Los Angeles County; no organics or cultivation; excellent orchard	
	pH	Moisture percentage	pH	Moisture percentage	pH	Moisture percentage	pH	Moisture percentage
	Sample 2-3 feet; moisture equivalent 19.3		Sample 0-0.5 foot; moisture equivalent 29.2		Sample 1-2 feet; moisture equivalent 25.6		Sample 1-1.5 feet; moisture equivalent 14.5	
0	3.70	2.6	3.94	5.9	3.70	3.7	....	....
0	4.52	2.7	3.96	6.0	4.00	3.7	....	....
0	4.61	2.7	3.96	6.1	4.00	3.6	....	....
0	4.50	2.7	4.00	6.0	4.00	3.5	....	....
0	....	....	....	....	....	....	....	....
.5	....	....	....	....	4.50	4.1	....	....
.5	....	....	....	....	4.62	4.1	....	....
.5	....	....	....	....	4.52	4.1	....	....
1.0	....	....	....	....	....	....	....	....
1.5	6.00	3.9	4.80	7.4	....	....	....	....
1.5	6.20	4.0	4.70	7.4	....	....	....	....
1.5	6.22	4.0	4.72	7.3	....	....	....	....
1.5	6.19	4.1	4.76	7.3	....	....	....	....
2.5	6.49	4.9	5.20	8.3	....	....	5.10	3.3
2.5	....	....	....	....	....	....	5.01	3.3
2.5	....	....	....	....	....	....	4.99	3.3
2.5	....	....	....	....	....	....	5.05	3.4
10.0	....	....	....	....	....	....	5.48	10.6
Samples at various depths								
			dry area	wet area	dry area	wet area		
500*	7.63	....	8.00	7.80	....	....	7.25	....
500	7.97	....	8.47	7.79	....	....	7.20	....
500	8.52	....	{8.07†	{7.58†	....	....	{5.70†	....
			{8.18	{7.58	....	....	{6.37	....
500	8.67	....	....	....	....	....	{6.52	....
			....	....	....	....	{6.63	....
500**	8.76	....	....	....	....	....	{6.93	....
			....	....	....	....	{6.90	....
Field*	7.27	24.8	5.63	6.40	13.8	29.7	6.11	23.5
Field	7.15	22.3	5.65	6.20	11.0	27.1	5.96	22.4
Field	7.11	29.2	{5.74	{5.90	{14.8	{27.1	{5.42	{11.8
			{5.75	{5.80	{16.1	{26.5	{5.54	{11.5
Field	7.10	16.6	....	....	....	....	{5.96	{10.9
			....	....	....	....	{6.10	{10.8
Field**	7.42	18.0	....	....	....	....	{6.15	{10.9
			....	....	....	....	{6.23	{8.9

\* \*\* Depth (feet): 0-0.5, 0.5-1, 1-2, 2-3, and 3-4, respectively.

† Foot divided into 6-inch fractions.

responding values determined in a similar manner for the wet area samples. The undiluted field samples in the wet area gave higher pH values than those in the dry area.

The data reported in table 12 show the effects of moisture on the pH of soils of orange orchards of various ages. All orchards except that at San Fernando were irrigated by means of low sprinklers. In the Placentia orchard, basin irrigation had been practiced until the pH determinations were made. The use of portable low sprinklers has enabled a better control of the application of the irrigation water and has already resulted in an appreciable decrease in the severity of chlorosis. The soil from this orchard showed a distinct alkalinity at the field moisture content but reacted acid at very low moisture percentages, as seen in table 12.

A marked acidity was found in the field determinations of the pH of the soils in the last three orchards considered in table 12. In the soil from the second orchard a variation of 4 pH units was brought about by varying the moisture content. In the fourth orchard, samples of soil were taken at 6-inch increments during the pH determinations made in the field. Below the first 6 inches, there was a gradual increase in the pH with each increment of 6 inches in the depth of the sample.

A second lot of soil was taken from the original field sample from the Rancho Santa Fe area (table 12), and was thoroughly mixed after being run through a 2-mm. sieve. Distilled water (3.5, 4.5, 5.5, 6.5, 7.5, 8.5, and 9.5 cc., respectively) was added to 100 gm. of the air-dried soil. The pH readings of the resulting mixtures were: 5.26, 5.62, 5.80, 5.92, 6.00, 6.10, and 6.17, respectively. The moisture percentages of these mixtures at the end of the tests were: 9.8, 11.0, 11.9, 12.5, 13.7, 15.1, and 16.3, respectively. Thus, the continued rise in the moisture percentage was accompanied by an increase in the pH readings.

In a comparison of soil samples from the wet and dry areas in the Rancho Santa Fe orchard, the pH values for the 1:5 soil-water suspensions were higher for the dry area, whereas the pH values of samples at field moisture were higher for the wet area. These data for a Valencia orange orchard are in agreement with those shown in table 11 for an avocado orchard at Escondido.

Several soil types varying widely in moisture equivalent percentages were selected as representative of many lemon orchards in southern California. The results of pH determinations on these soils are given in table 13. Many of the pH readings are typical of these soils below or near the wilting point. The sagebrush covered soil adjacent to the lemon orchard at Fallbrook became more acid as the depth increased as the parent rock was approached, even though the moisture percentage was increasing with depth. In the case of the soil from Upland it was necessary to screen out the rocks before any sample could be obtained. The soils from Ventura County tended toward alkalinity at the field moisture content. At the 1:5 soil-water ratio the Ventura soils were distinctly alkaline, but at very low moisture contents they reacted acid.

The results in the tables thus far make it appear that at very low moisture

contents, all soils may be on the acid side of neutrality. The data presented in table 14 show that this is not entirely true. In alkaline soils the increase in pH with increases in the soil-water ratio is considered to be due to the

TABLE 13  
*Effect of moisture content on pH values of soils from lemon orchards*

DISTILLED WATER, CC., ADDED TO 100 GM. OF AIR-DRIED SOIL	HANFORD STONY SANDY LOAM; UPLAND IN SAN BERNARDINO COUNTY; COMPLETE FERTILIZER; NO MANURE OR COVER CROP; EXCELLENT ORCHARD		VISTA SANDY LOAM; SAGEBRUSH COVERED LAND ADJACENT TO A LEMON ORCHARD; FALLBROOK IN SAN DIEGO COUNTY		YOLO GRAVELLY LOAM; BORON AND CHLOROSIS- AFFECTED ORCHARD; FILLMORE IN VENTURA COUNTY		SORRENTO SANDY LOAM; BORON AND CHLOROSIS- AFFECTED ORCHARD; OXNARD IN VENTURA COUNTY		SORRENTO CLAY LOAM; MONTALVO IN VENTURA COUNTY	
	pH	Moisture percent- age	pH	Moisture percent- age	pH	Moisture percent- age	pH	Moisture percent- age	pH	Moisture percent- age
	Sample 0-0.5 feet; moisture equiv- alent 11.2		Sample 2-3 feet; moisture equiv- alent 16.7		Sample 2-3 feet; moisture equiv- alent 18.7		Sample 1-2 feet; moisture equiv- alent 23.2		Sample 2-3 feet; moisture equiv- alent 27.8	
0	....	....	....	....	4.50	1.9	....	....	....	....
0	....	....	....	....	4.77	1.7	....	....	....	....
0	....	....	....	....	4.80	1.7	....	....	....	....
0	....	....	....	....	4.83	1.8	....	....	....	....
0	....	....	....	....	....	....	....	....	....	....
0.5	....	....	....	....	....	....	....	....	....	....
0.5	....	....	....	....	....	....	....	....	....	....
0.5	....	....	....	....	....	....	....	....	....	....
1.0	....	....	....	....	....	....	....	....	....	....
1.5	....	....	....	....	6.40	3.2	....	....	....	....
1.5	....	....	....	....	6.48	3.1	....	....	....	....
1.5	....	....	....	....	6.46	3.1	....	....	....	....
1.5	....	....	....	....	6.51	3.1	....	....	....	....
2.5	5.63	3.3	5.33	4.8	6.92	4.0	6.50	4.9	6.02	5.5
2.5	5.70	3.2	5.58	4.9	....	....	6.45	4.9	6.25	5.5
2.5	5.74	3.3	5.50	4.9	....	....	6.46	4.9	6.39	5.7
2.5	5.77	3.3	5.50	4.8	....	....	6.50	4.8	6.34	5.6
10.0	6.12	10.5	6.37	12.3	....	....	7.39	12.3	7.20	13.3
Samples at various depths										
500*	6.48	....	7.00	....	8.03	....	8.40	....	7.36	....
500	6.20	....	7.14	....	8.48	....	8.86	....	7.40	....
500	....	....	6.83	....	8.58	....	8.89	....	8.13	....
500	....	....	6.98	....	8.60	....	8.90	....	8.37	....
500**	....	....	....	....	8.33	....	8.82	....	8.51	....
Field*	5.77	13.4	6.13	6.6	7.13	16.9	7.56	11.8	6.25	20.9
Field	5.56	11.6	6.30	7.9	7.44	16.8	7.59	18.3	6.50	21.0
Field	....	....	6.03	11.0	7.39	12.8	7.54	16.6	6.70	22.8
Field	....	....	5.84	13.5	7.15	12.8	7.55	19.3	7.07	24.6
Field**	....	....	....	....	6.93	10.9	7.59	24.1	7.08	26.7

\*..\*\* Depth (feet): 0-0.5, 0.5-1, 1-2, 2-3, and 3-4, respectively.

hydrolysis of the potentially alkaline salts and minerals in the soils (1, 5). As shown in the tables, many of the soils show an increase in pH not only with the increase in soil-water ratio on the alkaline side of neutrality but also with lower soil-water ratios on the acid side of neutrality. This would indicate that when these soils are on the acid side of neutrality they contain potentially alkaline salts. Very little is known regarding the pH relation of many salts found in soils of low moisture percentages.

*Effects of low moisture percentages in soils covering a wide range of pH*

Leftovers, from a study<sup>3</sup> relative to the uniformity of pH readings of soils at high and low moisture percentages, consisting of very small soil samples

TABLE 14

*Effect of low moisture percentages in soils covering a wide range of pH*

SAMPLE NUMBER*	FIELD MOISTURE CONTENT		pH AT 1:5 SOIL-WATER RATIO	MOIS-TURE EQUIV-ALENT OF SAMPLES	MIXTURE OF AIR-DRY SOIL AND DISTILLED WATER									Mois-ture per-cent-age at end of test
	Dis-tilled water added to 100 gm.† of dry soil	pH			Air-dry soil	Dis-tilled water	pH values obtained by the consecutive use of the same initial sample							
	cc.*			per cent	gm.	cc.								
9	55.0	5.49	5.78	....	29	1.5	5.24	4.93	4.82	5.00	4.88	4.97	6.9	
1	32.5	7.56	8.42	20.3	100	2.5	6.56	6.62		6.59	.....	.....	5.3	
7	45.0	7.50	7.99	25.9	75	1.5	6.50	6.50		6.50	.....	.....	4.4	
4	17.5	7.67	8.32	10.1	79	1.5	6.87	6.80			.....	.....	2.2	
8	45.0	7.71	8.10	26.5	75	1.5	6.63	6.70			.....	.....	4.3	
6	35.0	7.87	8.40	23.6	75	2.0	6.81	6.81			.....	.....	4.4	
5	32.5	9.66	10.36	33.6	93	1.5	7.23	7.20			.....	.....	3.0	
3	20.0	9.89	10.34	....	75	1.5	8.50	8.49			.....	.....	2.2	
2	32.5	10.05	10.43	17.2	79	1.5	8.60	8.60			.....	.....	3.1	

\* Data kindly supplied by the University of Arizona Agricultural Experiment Station.

† Only 20-gm. samples were used in actual practice.

covering a wide range of pH, were utilized in pH studies at extremely low moisture contents, even though the origin of these particular soils was unknown. The data are reported in table 14. The moisture equivalent was determined for those soils the samples of which were adequate. Sample 3 puddled so badly that the duplicate determinations were too far apart to be considered reliable; the other duplicate determinations were in very close agreement. In most cases in table 14, the amount of water added in order to secure the field moisture content far exceeded the amounts necessary to bring the soils to

<sup>3</sup> It was a pleasure to serve as one of the analysts participating in this study, recommended by the Palo Alto meeting of the Western Society of Soil Science and undertaken by Dr. W. T. McGeorge, of the University of Arizona Agricultural Experiment Station, to whom thanks are due for these useful samples.

their moisture equivalent percentages. Nevertheless, the soils represent a wide range of pH, whether the pH values at the field moisture content or at the 1:5 soil-water ratio are considered. Since not more than 100 gm. of each original soil sample was on hand, the following method was employed: After the determination of pH at an extremely low moisture content, the sample was removed from the beaker and was stirred again as though it were a new sample; the regular procedure for determining the pH was then repeated. In this manner, as seen in table 14, satisfactory checks were obtained with a given sample. With sample 1, when no distilled water was added, it was not possible to obtain satisfactory checks, the values being 6.52, 6.12, and 6.72; when 2.5 cc. of water was added, the values were very nearly uniform, as shown in table 14. As the last column indicates, satisfactory pH readings were obtained over a wide range of pH with very low moisture percentages extending from 2.2 to 6.9. Even at moisture percentages as low as 2.2 to 3.1, samples 5, 3, and 2 still showed considerable alkalinity. Samples 1, 7, 4, 8, and 6, which were alkaline at the field moisture content, gave pH readings on the acid side of neutrality when the moisture content was greatly lowered.

In many orchards there are areas between the trees in the row in which the soil may be unirrigated and therefore of low moisture percentage. Although such areas most likely have very much lower pH values than the wetted areas, the extent or quality of root activity in them is still open to question. It is common observation that in soils of high pH and in which the calcium carbonate content and the moisture percentages are high, the trees improve materially and become darker green in the late spring as the soil moisture is brought progressively closer to depletion. On the other hand, the drying out of the entire soil mass to varying depths and degrees has been accompanied in many cases by severe fruit drop and other consequences such as excessive salt concentration. In many orchards today, some form of alternately drying and wetting certain localized portions of the soil is practiced with good results. In the orchard such practice at the same time provides for adequate soil aeration. Aeration, availability of soil nutrients or deficiency diseases, root disease, salinity, and many other factors that affect the health of orchard trees may be directly or indirectly related to the pH of the soil. What that pH may be and how it is affected, related, or controlled, are problems in the solution of which the results reported here form an integral part.

#### SUMMARY

The pH of soils at very low as well as at higher moisture percentages may be determined satisfactorily by means of the glass electrode. Experiments with purified quartz sands in conjunction with buffer solutions have shown that the pH values obtained in a several-phase system, when the determinations are properly conducted, are directly comparable to the results obtained in aqueous solutions.

The importance of the glass electrode contact with the soil, especially when

low moisture percentages are involved, is stressed in obtaining the greatest degree of accuracy in pH studies. The glass electrode is capable of giving equilibrium pH values; whether the equilibrium observed is identical with the equilibrium sought depends upon the investigator.

Th moisture percentages are shown to affect very markedly the pH values of soils. Very few, if any, orchard soils continued to react alkaline when the moisture content was sufficiently reduced.

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# A COMPARISON OF VARIOUS EXTRACTING SOLUTIONS FOR MEASURING THE AVAILABILITY OF PHOSPHORUS IN SOILS OF KNOWN FERTILIZER TREATMENT AND CROP PERFORMANCE<sup>1</sup>

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In carrying out extensive investigations of the effects of long-continued phosphorus fertilization of Kentucky soils, a number of acid, base, and salt solutions were used in an effort to extract definite fractions of the soil phosphorus and to evaluate their usefulness in measuring the soil phosphorus which is available to crop plants. It is the purpose of this paper to present data on this latter phase of the study.

A number of investigators have reported work of this kind recently, particularly in connection with results from the use of quick-test methods, but relatively few have dealt with both limed and unlimed plots that have been treated for long periods with both rock phosphate and the superphosphates, and few have compared different extractants under similar conditions. Ford (4), in his study of the availability and nature of the phosphates in certain Kentucky soils, made a start in this direction by using two extractants, sulfuric acid and carbonic acid. Some comparisons have been made by Hibbard (5), who discusses the results obtained by fourteen chemical and six biological methods as applied to calcareous soils, and De'Sigmond (9) has summarized the results from the use of different extractants on soils in general.

It is generally believed that the best laboratory method for estimating the relative ability of noncalcareous soils to supply phosphorus to crops is the one involving extraction with dilute acid solutions. The amount of phosphorus extracted from the soil by such a solution may be affected greatly by certain factors not related to the actual solubility of the element or its availability to the crop. These factors include, among others, the reaction of the soil, the form in which the phosphorus is held, the ability of the soil to refix the dissolved phosphorus in the presence of the extracting solution, and the nature of the solution itself. An attempt has been made to evaluate some of these factors under Kentucky soil conditions.

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station; the paper is published by permission of the director.

## CHEMICAL METHODS

*Total phosphorus* was determined by either one of two methods: (a) the A. O. A. C. magnesium nitrate method, or (b) fusion with sodium carbonate, phosphorus being determined in an appropriate aliquot by the colorimetric method of Truog and Meyer (11). The two methods agree very well, though the latter tends to give slightly higher results.

*Extraction of phosphorus* was for the most part conducted by shaking a weighed amount of soil (usually 1 to 5 gm.) with a measured volume (200 cc.) of the extracting solution at approximately 5-minute intervals for 1 hour, and filtering on a phosphorus-free paper. When organic acids and alkaline solutions were used the time period was extended to from 4 to 8 hours. Aliquots of these extracts were treated in such a manner that phosphorus could be readily determined in them by the colorimetric method. Values for the most part are averages of duplicate determinations.

*Reaction of soils and solutions* was determined by the glass electrode method.

## SOILS USED

The soil samples used in this study were collected from plots on six of the Kentucky soil experiment fields, designated as the Berea, Greenville, Princeton, Campbellsville, Mayfield, and Lexington. All the soils are of residual origin, those from the first four fields being derived from sandstone and shale, that from Mayfield from loess, and that from Lexington from the highly phosphatic Trenton limestone; the shale from which the Campbellsville soil is derived contains considerable calcareous material. The Greenville field is located on Tilsit, the Mayfield on Grenada, and the Lexington on Maury silt loam. The other fields have not been mapped, and the soil series to which they belong are not definitely known, though each is typical of the region and is fairly well drained. All are nearly uniform in texture—the clay content ( $<.002$  mm.) of four of the soils varies between 14.0 and 16.7 per cent, and the silt between 36.8 and 47.0 per cent—as determined by the international pipette method.

Surface soil samples were taken from each plot to the depth of the plowed layer. The Berea field was sampled in the fall of 1937, and all the rest were sampled in the fall of 1938. The samples were placed in clean cotton bags, air-dried on a greenhouse bench, passed through a 20-mesh screen, and stored in waxed paper containers. The portion that did not pass through the screen was weighed and discarded.

The crops grown on all the fields except Mayfield and Lexington show a very great response to phosphate fertilizers; those at Mayfield show a moderate response, and those at Lexington none. Corn, wheat, and mixed hay crops are grown on the Berea, Greenville, Campbellsville, and Mayfield fields in 3- or 4-year rotations. On the Lexington field, alfalfa is substituted for the mixed hay, and at Princeton a permanent pasture is maintained with mixed grasses.

Since the establishment of these fields, various fertilizers have been added to certain of the plots. Manure was used on all the plots most of the time, in amounts in proportion to the crop removed. Since the fields were established, the limed plots have received from 4 to 5.75 tons of ground limestone per acre, mostly in the first two rounds of the rotation. Superphosphate and

TABLE 1  
*Phosphorus added and total phosphorus found in the soil of variously treated plots*  
Pounds per 2,000,000 of air-dry soil

FIELD	YEARS OF CROP-PING	M*	MSP		MRP		MLSP		MLRP	
		P con- tent	P added	P con- tent	P added	P con- tent	P added	P con- tent	P added	P con- tent
Berea.....	24	600	237	650	890	1210	237	724	890	1226
Greenville.....	24	616	237	750	890	1290	237	796	890	1222
Mayfield.....	24	832	237	1006	890	1492	237	1020	890	1620
Campbellsville.....	18	684	251	744	944	1166	257	876	944	1270
Princeton.....	10	.....	...	...	262	742	70	780	...	...
Lexington.....	27	6700†	...	...	...	...	...	...	...	...

\* In this table and subsequently, the following abbreviations are used: M = manure; SP = ordinary superphosphate; RP = rock phosphate; L = ground limestone; R = residue.

† R.

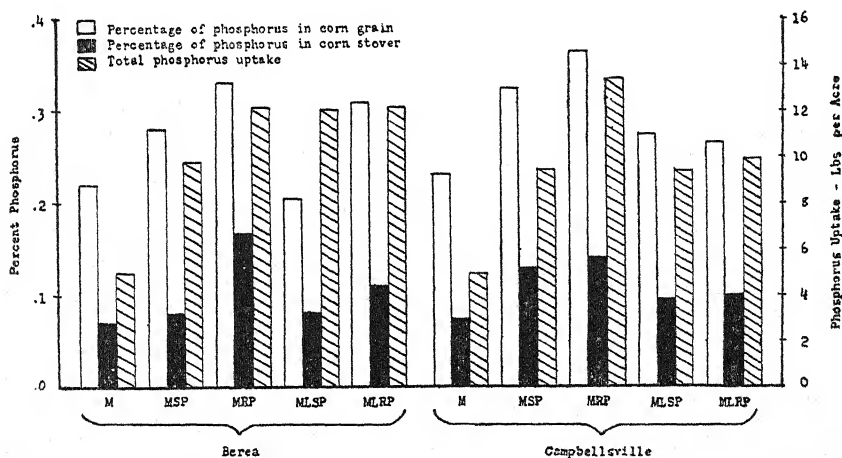


FIG. 1. PHOSPHORUS CONTENT OF CORN CROP

rock phosphate were applied once in each rotation. Full details of fertilizer and cropping practices are given by Roberts, Freeman, and Kinney (8). The plot from the Lexington field received no fertilizers, but crop residues were returned each year. The total amount of phosphorus applied to the treated plots of the respective fields are given in table 1, together with the present total phosphorus content as determined by analysis. It can be seen that the two amounts are somewhat proportional.

The use of phosphate fertilizers on all these fields, except the Lexington, has increased not only the total phosphorus content of the treated plots but also the crop yields and the percentage of phosphorus in the crops themselves. These increases were effective immediately. In most cases the use of limestone with phosphorus has further increased yields, though in some instances, particularly during the first years of the experiment, the use of limestone together with rock phosphate actually reduced the yield below that where rock phosphate was used alone. The average annual yields of dry matter on all the plots for the years from 1935 to 1938 inclusive are given in table 6. The data show the effect of ground limestone and phosphate fertilizers on the productivity of the soils. The data for the Princeton field are not directly comparable with those of the other fields because they represent yields of the pasture grasses as determined from cage clippings. The phosphorus content of the crop grown on these plots was in all cases increased over the check plots, but this increase is more marked in the crops from the unlimed plots than in those from the limed plots. In figure 1 data are presented showing the percentage and total uptake of phosphorus in the corn crops grown on the Campbellsville and Berea fields. The data are averages of two crops (1937-38) and two kinds of corn in the case of the Campbellsville plots, and only one crop (1938) and two kinds of corn in the case of the Berea plots.

#### EXTRACTION OF EASILY SOLUBLE PHOSPHORUS

When this work was started, it was convenient to use an equilibrium extraction method and shake the suspension of soil and solution in flasks by hand. To check on this method of shaking as compared with mechanical shaking, soils from two of the fields were compared. One group was shaken by rotating the flasks vigorously for half a minute or so ten times during 1 hour. The other set was shaken continuously for 30 minutes in a reciprocal shaking machine. In both cases 1 gm. of soil was shaken with 200 cc. of solution. Results are given in table 2 for a number of soils and for two different acid concentrations.

The data show that the two methods give similar though not identical results. More phosphorus was removed in some cases by hand shaking than by mechanical shaking, undoubtedly because of the longer time interval in the hand-shaking process. Where the weaker acid was used, the continuous shaking process removed more phosphorus than did hand shaking. This is probably because the more vigorous agitation kept fresh solution of the weaker acid in contact with the rock phosphate in these soils.

Another factor, besides thoroughness of shaking, in keeping the solution and solid particles in intimate contact is fineness of grinding. This, however, may also have some effect on the refixation of dissolved phosphorus by the soil before removal by filtration. Twenty-mesh soil was used for most of the work herein reported, but it seemed desirable to determine the effect of par-

ticle size on the amount of phosphorus removed. By prolonging the extraction period, it might also be possible to determine to what extent refixation of the released phosphate occurs in the soil in contact with the acid solution used. Portions of the Berea soil were ground to pass through 10-, 20-, 40-, 60-, and 100-mesh screens. Samples were extracted with  $H_2SO_4$  solutions of different strengths and for different periods of time with occasional hand shaking. The results, shown in table 3, indicate that the rate of solution is influenced by fineness of grinding, particularly with the weaker acids, though the differences are not great for soils ranging between 10 and 60 mesh. The tendency of this soil to fix phosphorus from acid solution as a result of prolonged contact with the extractant is not great except in the few instances

TABLE 2  
*Phosphorus extracted from soil by hand and by mechanical shaking*  
Pounds per 2,000,000 of air-dry soil

LABORATORY SOIL NUMBER*	HAND SHAKING 60 MINUTES	MECHANICAL SHAKING 30 MINUTES
0.05 N $H_2SO_4$		
39	58	64
41	560	590
43	66	70
45	600	700
47	20	12
0.1 N $H_2SO_4$		
655	36	30
669	38	30
779	24	20
781	520	460
783	480	430

\* Soil experiment fields, plot numbers, and fertilizer treatments of soils listed in this and subsequent tables are given in table 6.

where 72-hour extraction yielded less phosphorus than 12-hour or shorter periods of extraction.

For comparing the action of various extractants other than water and possibly certain salt solutions above pH 4.5, a single extraction is believed to be sufficient. Hibbard (6), using water-extraction methods, has found it desirable to use up to seven successive extractions. By this means he has studied the ability of the soil to supply phosphorus to the soil solution.

When acid-extraction methods are used and particularly where the acid is stronger than 0.002 N, one or two extractions reduce the soluble phosphorus of these soils to a level where further extractions, except in the case of the

Maury soil, give values that tend to approach a constant, and further extraction is of little value. Table 4 shows the effect of successive extractions on the amount of phosphorus removed from the Berea, Campbellsville, and Lexington soils.

TABLE 3

*Phosphorus extracted from Berea plots, as affected by fineness of grinding and length of extraction period*

Pounds per 2,000,000 of air-dry soil

LABORATORY SOIL NUMBER	A.S.T.M. SCREEN SIZE	0.002 N H <sub>2</sub> SO <sub>4</sub>				0.05 N H <sub>2</sub> SO <sub>4</sub>		
		5 min.	30 min.	12 hr.	72 hr.	5 min.	30 min.	12 hr.
39	10		18	22	22	...	...	...
41		150	146	292	412	...	...	...
43		12	20	32	32	...	...	...
45		240	260	328	400	...	...	...
47		5	7	7	7	...	...	...
39	20	10	18	26	28	...	44	72
41		110	210	300	356	490	514	580
43		14	20	32	26	54	64	70
45		136	230	360	420	500	560	554
47		8	10	9	6	10	12	18
39	40	16	22	28	30	50	74	80
41		110	206	330	436	464	520	580
43		16	24	28	22	68	74	94
45		170	270	370	460	504	600	620
47		7	10	7	7	10	12	20
39	60	20	28	30	40	62	68	88
41		154	244	368	400	512	560	584
43		22	26	32	36	62	74	90
45		226	304	420	480	...	650	654
47		10	11	10	6	14	18	26
39	100	40	60	52	56	64	76	114
41		240	400	460	520	540	580	580
43		42	52	52	54	72	90	120
45		276	406	450	440	600	620	...
47		11	14	10	7	22	28	36

The effect of varying the ratio of soil to solution is of considerable importance not only in measuring the availability of phosphorus but also to some extent as an index of the ability of a soil to fix phosphorus, and under certain conditions, as pointed out by Truog (12), in estimating the compounds in which most of the phosphorus is tied up. Table 5 gives the results of varying the soil-solution ratio between 1:800 and 1:10.

The results indicate that there was a gradual decrease in the amount of

phosphorus dissolved as the amount of soil increased, but the ratio had little effect on the relative amount of phosphorus extracted from the different soils. The decrease is due either to the time and method of extraction or to a certain

TABLE 4

*Phosphorus removed from soils by successive 1-hour extractions with dilute  $H_2SO_4$*   
Pounds per 2,000,000 of air-dry soil

LABORATORY SOIL NUMBER	0.05 N $H_2SO_4$				0.1 N $H_2SO_4$		
	Number of extraction				Number of extraction		
	1	2	3	4	1	2	3
39	64	19	10	8	66	46	20
41	524	64	22	...	560	74	24
43	62	19	12	...	66	40	20
45	564	58	20	12	....	...	...
47	18	8	4	4	....	...	...
655	30	10	6	6	....	...	...
669	30	10	8	6	....	...	...
779	16	6	4	4	....	...	...
781	500	42	20	12	460	42	20
783	460	44	20	8	440	52	20
192	1440	432	340	204	1850	770	284
182	....	...	...	...	120	22	8
183	....	...	...	...	32	44	26

TABLE 5

*Phosphorus extracted from soils with 0.05 N  $H_2SO_4$  as affected by soil-solution ratio*  
Pounds per 2,000,000 of air-dry soil

LABORATORY SOIL NUMBER	1/800	1/400	1/200	1/40	1/20	1/10
39	64	66	58	62	54	36
41	540	560	550	530	465	390
43	64	66	64	62	54	36
45	550	560	580	464	475	424
47	32	24	20	18	15	12
655	44	38	32	30	26	18
669	38	....	28	30	26	18
779	32	24	14	14	14	14
781	490	490	500	500	425	360
783	420	400	420	448	380	320
192	1880	1840	1440	1440	1240	920

amount of refixation of dissolved phosphorus in the acid solution. Both factors probably affected the results obtained. For practical purposes of measuring chemically available phosphorus, it seems probable that any of the ratios would be satisfactory in soils not having a considerably higher fixing power than these.



TABLE 6

Data for soils from different fields, including plot treatment, pH, phosphorus content, yields, and amounts of phosphorus extracted with different solvents  
Phosphorus in pounds per 2,000,000 of air-dry soil

PHOSPHORUS EXTRACTED																				
LABORATORY SOIL NUMBER	PLOT NUMBER	TREATMENT	pH	TOTAL P CONTENT	YIELD (LBS.) AVERAGE YEARLY TOTAL DRY MATTER (1935-1938)	OR-GANIC P*	Bray's test 0.75 N HCl†	H <sub>2</sub> SO <sub>4</sub>			Acetic acid 5%	0.5 N NH <sub>4</sub> -acetate (pH 5.0)	Oxalic acid 1%	Oxalic 0.25% 0.01 N HCl	Tar-taric acid 0.5%	Citric acid 1%	Buf-fered HClO <sub>4</sub> 0.75% + NaClO <sub>4</sub> 25%	0.2 N Na <sub>2</sub> CO <sub>3</sub>	0.25 N NaOH	
								0.002 N	0.02 N	0.05 N										
																				0.1 N
<i>Berea</i>																				
47	308	M	4.85	600	1578	166	V L	12	18	20	24	5	104	54	6	10	27	75	97	
39	304	MSP	4.8	650	2301	230	L	24	46	58	76	10	208	114	18	20	28	81	125	
41	305	MRP	5.05	1210	2988	240	V H	220	450	550	600	14	460	600	200	344	275	154	165	
43	306	MLSP	5.77	724	3483	270	M	26	52	64	78	6	142	108	18	20	35	112	132	
45	307	MLRP	5.95	1226	3754	220	V H	270	510	590	650	20	420	568	180	460	245	86	120	
<i>Greenville</i>																				
333	403	M	5.08	616	2012	160	V L	8	12	12	16	3	...	36	4	...	...	...	90	
335	404	MSP	4.90	750	3692	230	V L	14	24	28	40	7	...	72	10	...	...	...	125	
339	405	MRP	5.35	1290	4705	310	V H	210	410	470	480	18	...	500	150	...	...	...	175	
345	407	MLSP	5.70	796	4310	280	V L	14	24	28	40	5	...	94	6	...	...	...	120	
349	408	MLRP	5.98	1222	4420	260	V H	235	390	410	492	16	...	440	136	...	...	...	120	
<i>Mayfield</i>																				
493	407	M	5.33	832	4755	284	V L	32	30	38	50	9	...	90	13	...	...	...	158	
485	404	MSP	5.12	1006	5257	200	M	40	72	80	100	14	...	146	28	...	...	...	200	
487	405	MRP	5.36	1492	6520	204	V H	260	460	480	580	26	...	620	180	...	...	...	210	
489	406	MLSP	5.98	1020	6970	240	M	40	78	80	100	18	...	138	24	...	...	...	165	
495	408	MLRP	6.12	1620	7290	264	V H	270	540	540	620	20	...	590	176	...	...	...	230	

*Campbellsville*

779	813	M	5.44	684	3920	240	V L	4	8	12	24	2	2	...	36	4	...	12	91	110
655	205	MSP	5.46	744	4893	236	V L	12	28	32	36	6	4	...	62	8	...	16	86	142
781	814	MRP	5.68	1166	6204	286	V H	204	490	500	520	196	10	...	410	128	...	250	92	145
669	212	MLSP	5.81	876	5723	294	V L	12	24	28	38	6	3	...	62	10	...	16	92	155
783	815	MLRP	5.98	1270	6344	280	V H	180	400	420	480	220	12	...	500	160	...	300	86	147

*Princeton*

181	1	O	5.4	...	2539	...	V H	14	...	...	10	...	...	...	...	...	...	...	37	48
182	2	RP	5.3	742	3121	200	V H	...	250	240	240	92	3	...	200	72	...	...	43	61
183	3	LSP	6.15	780	3196	230	V L	8	18	20	30	4	3	...	44	8	...	...	52	80

*Lexington*

192	310	R†	5.65	6700	6273	480	V H	1040	1200	1200	...	376	72	...	2272	352	...	...	...	925
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\* Method of Dean (3). † VL = very low, L = low, M = medium, VH = very high. ‡ = Residues returned.

The group of soils used was extracted with 25 different acid, base, and salt solutions and combinations. All of the acids and bases had been used previously by other workers for extracting soils, in many cases in connection with the development of rapid-test procedures. No attempt was made to follow the exact procedures as outlined by the respective users of each. For this reason the work reported here is not primarily a comparison of so-called "quick tests," but rather a comparison of different chemical solutions for dissolving phosphorus from soils variously treated with phosphate fertilizers. Not every solution was used at widely different concentrations or ratios of soil to solution, but enough trials of this nature were made to find the most satisfactory conditions for each. The pH of the soils ranged between 4.8 and 6.15, and in no case was the pH of the extract appreciably different from that of the initial solution. The data obtained from the use of some of these extracting solutions, together with the data for total phosphorus, organic phosphorus, and crop yield of each of the soils are presented in table 6.

#### DISCUSSION

In comparing the data obtained from the use of the different extractants, it was found that the mineral acids, sulfuric, hydrochloric, and nitric, gave results so nearly alike that there was little choice among them; the data for the last two, therefore, are not reported in table 6. Sulfuric acid lends itself a little better than the others to the colorimetric procedure used, and hence is more convenient. Acetic acid, citric acid, and perchloric acid buffered with sodium perchlorate acted in a manner similar to the mineral acids in removing slightly more phosphorus from soil of the limed than from that of the unlimed plots. Ammonium or sodium acetate, at a pH of 5.0 or below, shows less difference between the rock phosphate-treated soils and those receiving superphosphate but for comparing the soils in other respects seems no better than the acids. The alkaline solutions and those containing oxalic and tartaric acid, however, remove more phosphorus from soil of the unlimed plots than from the limed. Replacement of adsorbed calcium with neutral ammonium acetate had little effect on this order. From the data obtained, it seems that there is no best extractant for a soil treated with different phosphorus carriers if a quantitative technic is used. The Bray "quick test," used widely in the state by county agents, was not sensitive enough in some cases to distinguish between the check plots and those receiving superphosphate fertilizers. For the practical purpose of estimating the phosphate fertilizer needs of soils, experience gained with the use of any one of the extractants on a given soil is of more importance than the extractant itself.

Much of the work in determining chemically available phosphorus in soils has been done with the idea of correlating the results with crop yields and field response to fertilizers. The method used has been judged good or bad depending on the degree of correlation obtained. For this to be a valuable criterion, however, the available phosphorus must be the only factor limiting

plant growth, which is often not true even for phosphorus-deficient soils. The lack of correlation between crop yields and the results of quick tests has received much attention and perhaps has been overemphasized.

Burd and Murphy (1), who recently discussed this matter on more or less theoretical grounds, assert that "fertilizers are not so valuable that the most effective dosage must be predicted with a high degree of accuracy." On the other hand, they contend that if the results of the analysis are doubtful, a medium application on good land is good insurance, particularly where the crop is valuable.

It is apparent that the best that can be done with any extractant is to measure the general level of comparatively available phosphorus. Except in a relative sort of way, it is impossible to measure only that portion of the phosphorus available to plant roots. Attempts have been made to do this by using several successive extractions with very weak solvents such as water or carbonic acid. It is difficult to see how this procedure could accomplish its purpose, because plant roots are extended so rapidly that they probably feed largely on a fresh area for very small amounts of the element at any one time. It would seem better to use a stronger solution and remove a relatively large amount of the element, assuming that if enough is present the plant can obtain a sufficient supply from the soil.

For convenience in considering the data in table 6, only those values for chemically available phosphorus from the 0.05 *N* H<sub>2</sub>SO<sub>4</sub> extraction are used. It is obvious that these values are low for the check plots (manure alone) of the four fields, Berea, Greenville, Mayfield, and Campbellsville. This agrees with the phosphorus-deficient condition of these soils as shown by the low crop yields and by the marked increases in yield when phosphate fertilizers are applied. The chemically available phosphorus in the Lexington soil (the highly phosphatic Maury silt loam) is high, which also agrees with the lack of crop response to phosphate applications on this soil. The correlation, however, between the chemically available phosphorus and crop yields on the check plots of the first four mentioned fields, is only very general. Both the chemically available phosphorus and the yields are higher on the check plots at Mayfield than at the other fields, but the chemical data are the same for Greenville and Campbellsville, whereas yields are much higher at Campbellsville than at Greenville. The chemical data are higher at Berea than at Greenville or Campbellsville, whereas crop yields at Berea are lower than at Greenville and much lower than at Campbellsville. One reason for this, undoubtedly, is the content of replaceable calcium in the unlimed soils, which is somewhat more favorable for crop growth at Campbellsville than at Greenville and much more favorable at Greenville than at Berea. Furthermore, the internal drainage of the soil of the three fields is equally favorable for crop growth. In unpublished greenhouse data where these factors have been eliminated, as well as the possibility of nutrients other than phosphorus limiting crop growth, comparative figures for yield of wheat grown in soil

from the check plots of the Berea and Campbellsville fields are 34 and 24, respectively. For very precise use of the chemical data in relation to crop growth, a somewhat different scale would be necessary for different soils.

The manure-superphosphate treatment has increased both chemically available phosphorus and crop yields at all four fields; however, the degree of increase is not the same for the two quantities, particularly at the Mayfield and Campbellsville fields. The use of lime together with superphosphate increased the crop yields without consistently increasing the chemically available phosphorus. It will be noted that this is not entirely in agreement with results obtained by Ford (4), Snider (10), Cook (2), and others who found that the application of lime to soils increased by a considerable amount the chemical availability of both native and applied phosphates. The difference is probably due in part to the method of extraction, since the aforementioned workers used mechanical shakers for 4 or 5 hours. The rock phosphate treatments increased chemically available phosphorus very much more than crop yields. From the nature of the extracting procedure, this is bound to be true. Practical difficulties at present probably would prevent the development of a procedure which would measure more accurately the availability to a particular crop of this phosphate relative to other phosphates and at the same time be as reliable when used with ordinary untreated soils. The chemically available phosphorus for the plots treated with rock phosphate is necessarily a measure only to a small degree of the phosphorus available to a particular crop. It mainly measures the phosphorus that crops can take from the soil over a considerable period of time. Only knowledge of previous fertilizer treatment can obviate serious error in interpreting phosphate tests at this point. This is in line with the findings of Richer and White (7), who in their study of the correlation of chemically available phosphorus with crop yields assert that "when readily available phosphorus as measured by the Truog method is to be used as one index of fertility it is of the utmost importance to know the history of the soils in question, particularly as to the kind of phosphorus fertilizers that have been applied."

At several of the fields, the phosphate-treated plots were divided in 1920, and the treatment was continued on one half and discontinued on the other half. Data from the two halves of these plots at Berea, not reported in this paper, show that the residual effect of application of phosphates in increasing chemically available phosphorus persists for some time; how long, obviously depends on the amount of phosphate applied and the fixing power of the soil. Seven years after the phosphate treatments were discontinued, the chemically available phosphorus for these plot halves is still well above that for the check plots. In most cases, however, it is 10 to 15 per cent less than on the halves of the plots where the applications have been continued, though crop yields are still at approximately the same level on the two halves.

In several instances where the chemically available phosphorus and the crop yields are not in very good agreement, the higher crop yields are found to be

associated with a higher content of organic phosphorus in the soils, which affects crop growth more than it does chemically available phosphorus. Some of the discrepancies between the different fields may thus be explained in part.

It seems probable that as applied to the soils of Kentucky, no one extractant is much better than another. The choice of any one should be on the basis of convenience and experience. On the soils used here, 0.05  $N$   $H_2SO_4$  has proved most satisfactory. A considerable amount of work should be done with the particular extractant selected before any specific recommendations are made on the results obtained. The use of several different extractants on the same soil is not worth while for the purpose of diagnosing fertilizer needs, though it is beneficial in predicting the form in which the phosphorus occurs in the soil and the capacity of the soil to fix phosphorus. More than one extraction of the soil is unnecessary.

#### SUMMARY

Soil samples from the check plots and those plots receiving superphosphate and rock phosphate, both with and without limestone, of six of the Kentucky soil experiment fields, were extracted with 25 different acid, base, and salt solutions, to compare the usefulness of these solutions in measuring the availability of phosphorus in soil.

The conditions under which the extraction procedure was carried out were found to affect the results to a certain extent but generally not enough to change the relation of one extractant to the others. These conditions include method of shaking, length of extraction period, fineness of grinding, soil-solution ratio, and number of extractions.

The solutions used may be divided roughly into three groups, on the basis of their effect on the variously treated soils, as follows: (a) the mineral acids and their acid salts, together with acetic and citric acid; (b) oxalic and tartaric acids and mixtures of these with hydrochloric acid; and (c) the alkaline solutions. All of these removed less phosphorus from the soils of the check plots than from the fertilized plots and considerably less from the plots treated with superphosphate than from those treated with rock phosphate. If the action of these extractants is understood, especially that of the last two groups toward unlimed and limed soils, any of them will indicate the general level of available phosphorus. No one extractant appears to be appreciably better than another, and the choice of any one should be on the basis of convenience and experience. From the standpoint of directly correlating the results obtained from their use with plant growth, all have certain limitations, but there is a general relationship in this respect, as for example between soils that have not been fertilized and those that have received fertilizers over a considerable period. The results obtained from the use of at least some of the extractants would be helpful in making fertilizer recommendations for most of the soils of the state, though where soil types vary considerably, a different scale might be necessary for soils of these different types.

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# A DIRECT-READING FLOWMETER AND ITS USE IN RESPIRATION STUDIES WITH PLANTS<sup>1</sup>

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In the course of a series of investigations on root respiration it became necessary to design a flowmeter that would enable rapid and accurate measurements of slow rates of flow of small volumes of gases. As each culture vessel requires an individual flowmeter, the instrument had to be compact, sturdy, and inexpensive. To meet these special requirements, certain modifications were made of Benton's<sup>2</sup> differential manometer to produce a highly sensitive flowmeter with a direct-reading indicator. The advantages of this model over the conventional type are considered to be of sufficient importance to merit its use for experimental procedures in which a flowmeter having these features is desired.

The construction and principles of this apparatus are evident from part 2 of figure 1. The apparatus is essentially a manometer to measure the pressure difference on the two sides of an orifice caused by resistance of the constriction to the passage of a gas. Thus the passage of a gas from the inlet tube *A*, through the capillary orifice *C*, to the outlet tube *E*, creates a difference in pressure in the two arms (*B* and *D*) of the water manometer. Since one arm (*D*) dips into a reservoir (*R*) of relatively large volume, while the other arm (*B*) is above the water, the difference in pressure in the two tubes will be manifested by a rise in the water level in *D*, and the height to which the water level rises will be a direct measurement of this difference in pressure. Because of the relatively large volume of the reservoir, the zero reading of the manometer, which corresponds to the water level in the reservoir (*R*), will not be appreciably changed by the displacement of liquid into *D*, and the calibrations on *D* can be made in linear units to indicate cubic centimeters of gas flow per minute per centimeter of liquid rise in *D*.

The flowmeter is constructed of soft glass tubing having a diameter of 0.3 cm., and is about 45 cm. high with a base diameter of 5 cm. The capillary resistance tube (*C*) is 4 cm. long.

The safety bulb *S* has a capacity of 25 cc., which is also the volume of liquid in the reservoir (*R*). A constant zero setting in the reservoir is easily obtained

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

<sup>2</sup> Benton, A. F. 1919 Gas flowmeters for small rates of flow. *Jour. Indus. and Engin. Chem.* 11: 623-629.



by thrusting the end of tube *D* to the bottom of the reservoir bottle and partly filling the bottle with an accurately measured volume of indicator fluid. The apparatus can thus be dismantled, cleaned, and reassembled without affecting the accuracy of the calibrations.

The position of the capillary provides an added advantage in that wetting of the capillary by the indicator fluid is a rare occurrence with this model,

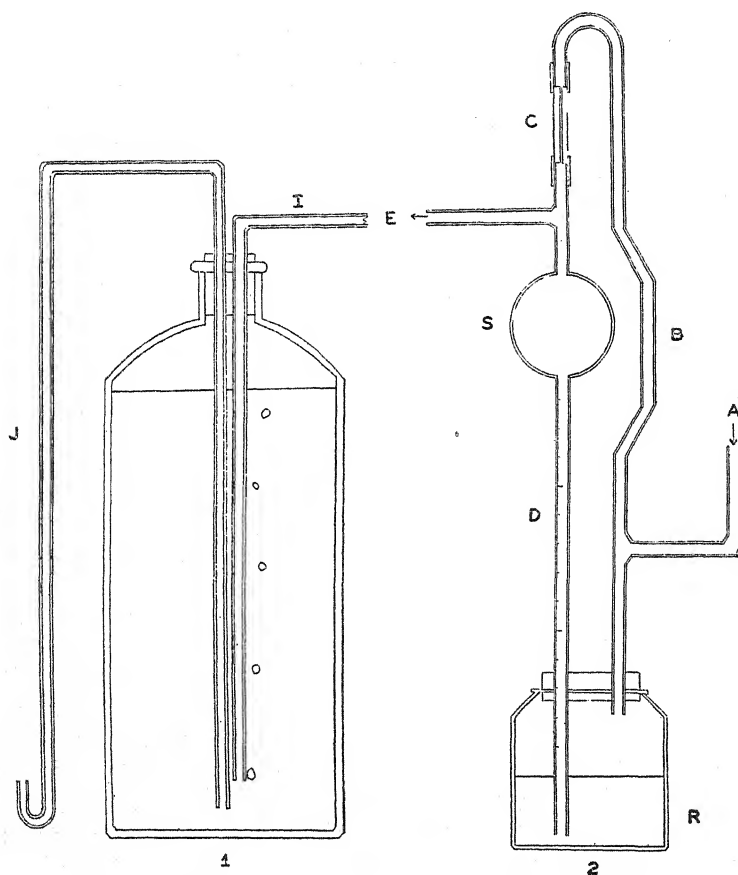


FIG. 1. DIAGRAM OF THE FLOWMETER AND THE GAS COLLECTOR

since any liquid that might accidentally overflow the safety bulb will be blown out through *E*. Reasonable care in making adjustments of the gas flow will prevent such accidents.

Loring<sup>3</sup> has recently described a direct-reading flowmeter using a reservoir

<sup>3</sup>Loring, A. D. 1939 Construction of manometers for measuring flow. *Indus. and Engin. Chem., Analyt. Ed.* 11: 626-628.

of this type. The apparatus here described is, however, of greater convenience in physiological work and is more easily constructed.

Since the entire unit is supported by the reservoir bottle (*R*), it can be easily transported to a new position, and its compactness is a distinct advantage in operation on a greenhouse bench where space is limited. Clean dry gases must be used, and all connections should be as short as possible. Rubber, free from sulfur bloom (nitrometer tubing), is used in the connections to the flowmeter. Any clogging of the capillaries by dust or water vapor will seriously affect the accuracy of the readings.

Calibrations over the range of flow desired are made by inserting a capillary (*C*) of the proper bore and measuring the rate of flow of gas with the indicator fluid at different marked levels in *D*. Three such calibrations are sufficient for the entire range of each flowmeter and capillary tube combination because of the linear relationship between rate of flow and indicator level in *D*. The sensitivity of the flowmeter will depend on the size (cross-sectional area) of the capillary bore (*C*).

Ratings of each capillary in cubic centimeters of gas flow per minute for any given water level in *D* can be made. For a wide range of gas velocities different capillaries are used, and the tube can be etched at integral units of gas flow when a permanent apparatus is required.

The method of calibration of the flowmeter involves certain features which have not been reported in the literature. The usual method of collecting the gas by displacement of water in a volumetric cylinder produces measurable changes in gas pressure under conditions of low rates of flow. Smith<sup>4</sup> partly overcomes this difficulty by measuring the water displaced by the gas, but even this method produces differences which affect the accuracy of the calibration. A constant level device was here used which will enable collection and measurement of the gas over periods as long as 24 hours with no change in back pressure.

Part 1 of figure 1 shows the essential features of this device to be a large bottle fitted with a two-holed rubber stopper with a gas inlet tube (*I*) reaching almost to the bottom of the bottle, and a siphon (*J*) of which the outlet point is level with the bottom of the inlet tube.

When such a bottle is filled with water, this will escape through the siphon when gas is forced in under even very low pressures, and only the rate of entering gas will determine the rate of water exit. The flowmeter is connected in series with the gas collector, the displaced water is caught in a burette, and the time required for the displacement of a measured volume is the measurement of the rate of gas flow.

Regulation of the rate of gas flow is most easily accomplished by a screw clamp or a stopcock at point *A*. For very low rates of flow a fine-bore capillary tube of the desired size should be inserted just behind the screw clamp or

<sup>4</sup>Smith, G. W. 1932 Apparatus for calibration of flowmeters. *Indus. and Engin. Chem., Analyt. Ed.* 4: 244-245.

stopcock. Such a capillary also aids in smoothing any pulsations which may occur in the gas flow.

In actual practice six flowmeters were calibrated at once by connecting them in series between the flowmeter (part 2) and the gas collector (part 1) and measuring the volume of water displaced in part 1. The resistance of the capillaries with a given main pressure is the only limit to the number of units that can be calibrated at one time. The more units in the series, the more time will be required to attain equilibrium of gas flow.

In the experiments which were carried out with this apparatus, the culture jar with growing plants occupied a position (*E*) between the flowmeter and the device which served to collect the gases for respiration measurements. When carbon dioxide is to be determined, its solubility in water requires its absorption before the gas mixture passes into the collecting device. For exact work with oxygen the water in part 1 of figure 1 is previously saturated with oxygen at its partial pressure in the gas mixture employed. The toxic effects of mercury vapor precluded the use of this liquid in the gas collector when working with living plants in the greenhouse.

Thirty of these flowmeter units were constructed and gave highly satisfactory results in connection with the quantitative measurement of gaseous exchange by the roots of living plants.

# OCCURRENCE OF RHIZOBIUM MELILOTI BACTERIOPHAGE IN SOILS

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The problem of the role of *Rhizobium* phage in nodules of, and in soils bearing, leguminous plants has been presented (1, 2, 4, 5, 7, 8) and requires no further elucidation. The present paper is a brief report of a survey of various soils in the Finger-Lake region of New York State with respect to the incidence of *Rhizobium meliloti* phage.

## EXPERIMENTAL

Soil samples were obtained from alfalfa fields on different soil types by means of augers which were carefully sterilized between samplings. Ten to twelve borings from soil in the immediate vicinity of the roots of the plants were thoroughly mixed and comprised one composite sample. Six hundred grams of soil from such a sample was added to 250 cc. of a yeast water medium [M5 of Laird (6)]; the mixture was inoculated with a suspension of a phage-susceptible alfalfa organism A2 (7) and was incubated for 3 days at 28°C.; it was then filtered through paper and the filtrate passed through sterile Berkfeld N candles. Three cubic centimeters of the resulting bacteria-free fluid was added to a suspension of the phage-susceptible organism, and observations for lysis were made every 24 hours for 3 days, after which the culture was filtered and 2 cc. of this filtrate tested in a similar manner. Serial transfers were thus continued until distinct dissolution was noted; if lysis did not occur after 10 passages, however, the filtrate was discarded. Lysis was usually obtained in the first transfer and was complete in the second. Controls were maintained by inoculating the susceptible organism into sterile medium and treating the resulting culture as those inoculated with soil. Soils from fields without legumes and from forested areas were also tested.

The original bacteria-free soil filtrates were also tested directly for phage by means of the plaque technic. One cubic centimeter of each of three dilutions (1:10, 1:100, 1:1000) of every filtrate was added to sterile Petri plates containing 1 cc. of a suspension of the susceptible organism; 10 cc. of yeast-water mannitol agar [M4 of Laird (6)] was poured into each plate, and the whole

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was thoroughly mixed and incubated at 28°C. Within 48 hours, plaques were visible on all plates with dilutions of 1:100 and 1:1000 of all the filtrates which

TABLE 1  
*Isolation of Rhizobium phage from soils planted to and free from alfalfa*

ALFALFA FIELD TESTED	AGE OF STAND	SOIL TYPE*	SAMPLE NUMBER	REACTION	PHAGE INCIDENCE	ALFALFA FIELD TESTED	AGE OF STAND	SOIL TYPE	SAMPLE NUMBER	REACTION	PHAGE INCIDENCE
	years			pH			years			pH	
1	1	Groton	1	5.58	+	10	5	Groton	36	5.26	+
			2	6.02	+	11	5	Lansing	37	6.28	+
			3	7.39	+				38	7.30	+
2	2	Lansing	4	6.16	+				39	6.89	+
			5	6.37	+				40	5.97	+
			6	6.02	+				41	6.30	+
			7	6.58	+	12	6	Groton	42	5.27	+
			8	6.66	+				43	5.88	+
3	2	Lansing	9	6.48	+				44	5.68	+
			10	6.87	+	13	6	Groton	45	5.61	+
			11	6.10	+				46	5.78	+
			12	6.40	+				47	5.56	-
			13	6.58	+	14	7	Volusia	48	5.86	+
4	3	Lansing	14	6.49	+				49	6.24	+
			15	6.63	+				50	6.52	+
			16	5.85	+				51	6.28	+
			17	6.49	+				52	6.58	+
			18	6.40	+	15	8	Lansing	53	6.22	+
5	3	Lansing	19	7.26	+				54	6.37	+
			20	6.78	+				55	6.10	+
			21	7.01	+				56	6.23	+
6	3	Groton	22	5.39	+	16	29	Lansing	57	6.72	+
			23	5.39	+				58	6.50	+
			24	6.78	-				59	6.31	+
7	4	Groton	25	6.04	+				60	6.72	+
			26	5.76	+				61	6.11	+
			27	6.03	+				62	6.24	+
8	4	Groton	28	5.92	+						
			29	5.77	-	Pasture		Dunkirk	63†	6.20	-
			30	5.77	+	Pasture		Dunkirk	64	5.27	-
9	5	Groton	31	5.48	+	Wheat		Dunkirk	65	6.11	+
			32	6.31	+	Forest soil			66	4.99	-
			33	5.39	-	Forest soil			67	4.90	-
10	5	Groton	34	5.63	+	Stored soil		Merrimac	68	4.51	-
			35	5.75	+						
						Control (medium 5 + susceptible organism)					-

\* All soils are silt loams of the series indicated.

† Soils 63-68 not planted to alfalfa.

were found by serial transfers to contain phage. This technic proved to be a rapid and useful confirmatory procedure.

The advisability of inoculating a soil-medium mixture with a phage-sus-

TABLE 2

*Incidence of Rhizobium phage in alfalfa plots on Caldwell Experimental Field\**

PLOT TESTED	AGE STAND	SAMPLE NUMBER	REACTION	PHAGE INCIDENCE
	<i>years</i>		<i>pH</i>	
1. Alfalfa.....	1	1	7.10	+
		2	6.98	+
2. Alfalfa.....	2	3	6.94	+
		4	7.10	+
3. Alfalfa.....	2	5	7.71	+
		6	6.31	+
4. Alfalfa + timothy.....	2	7	6.70	+
		8	6.14	+
5. Alfalfa.....	3	9	6.94	+
		10	5.94	+
6. Alfalfa.....	5	11	7.04	+
		12	6.68	+
7. Frame 1. Timothy and lime.....	10+	13	8.27	+
Frame 2. Alfalfa and lime until 1938.....	10+	14	8.21	+

\* Dunkirk silt loam.

TABLE 3

*Presence of phage in boxes containing soil inoculated with rhizobia and treated with various fertilizers\**

BOX NUMBER	TREATMENT	REACTION	RHIZOBIA PRESENT AT LAST SAMPLING†			PHAGE INCIDENCE
			Red clover	Alfalfa	Pea	
		<i>pH</i>				
1	Check	5.49	0	0	0	—
2	NaNO <sub>3</sub>	4.97	0	0	0	—
3	Na <sub>2</sub> CO <sub>3</sub>	5.22	0	0	0	—
4	KCl	5.21	0	0	0	—
5	CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub>	5.29	0	0	0	—
6	CaSO <sub>4</sub>	5.14	0	0	0	—
7	Sucrose	5.64	0	0	0	—
8	2,000 lbs. CaCO <sub>3</sub>	6.10	5,000	5,000	1,000	—
9	4,000 lbs. CaCO <sub>3</sub>	7.04	100,000	500	1,000	—
10	4,000 lbs. CaCO <sub>3</sub> + NaNO <sub>3</sub>	6.78	5,000	500	1,000	+
11	4,000 lbs. CaCO <sub>3</sub> + Na <sub>2</sub> CO <sub>3</sub>	7.24	1,000	10	10	+
12	4,000 lbs. CaCO <sub>3</sub> + KCl	6.33	10,000	10	10	+
13	4,000 lbs. CaCO <sub>3</sub> + CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub>	6.60	100,000	10	1	+
14	4,000 lbs. CaCO <sub>3</sub> + CaSO <sub>4</sub>	6.60	5,000	10	500	—
15	4,000 lbs. CaCO <sub>3</sub> + sucrose	7.14	5,000	1,000	10	+

\* No plants were allowed to develop in the boxes.

† Numbers per gram of soil.

ceptible strain prior to incubation may be questioned, as this treatment might conceivably stimulate the production of a homologous phage during incubation. A number of samples were accordingly tested for phage in the presence and in

the absence of such inoculum. The lytic agent was obtained regardless of inoculation. This practice, therefore, is not an essential one; nevertheless, it results in the activation of the lytic principle, thus making for greater ease of demonstration in a short time, and consequently it was always employed.

Phage was found in every alfalfa field tested and in 58 of the 62 samples taken (table 1), but there was no apparent correlation between age of alfalfa stand, soil type or reaction (between the pH limits of 5.26 and 7.39), and phage incidence. The lytic agent was isolated in only one instance from a soil not bearing legumes; it is possible that wind contamination and the growth of volunteer alfalfa plants were responsible for this.

The Caldwell Experimental Field of the New York State College of Agriculture contains plots bearing alfalfa stands varying in age. These plots were tested in the usual manner. Phage was found to be present in all the plots and in every sample taken (table 2). Again no relationship between age of field, soil reaction, and presence of the lytic principle could be demonstrated.

An experiment in progress at the Caldwell Experimental Farm on the longevity of rhizobia in soil as affected by fertilizer treatment provided material for further study. Representative species of rhizobia had been added in 1931 to the treated soil in boxes which were buried in the ground, and tests had been made frequently for the survival of the bacteria. The necessary information is summarized in table 3, along with pH values and the results of phage-incidence studies. The organisms in boxes 1-7 had disappeared 6 months after being introduced. This was due to the unfavorable reaction (3), since under conditions of higher pH the bacteria were present when last tested (1938) although there was little correlation between their survival and the treatment.

It appears that phage was present only where lime and certain soil amendments had been added [the presence of the lytic principle is hardly to be expected in boxes 1-7 because of the low pH (6)], but reaction alone was not the responsible factor. It is possible that certain fertilizer treatments are conducive to the development of the lytic agent at the expense of the organism introduced, but the evidence is too meager to support any definite conclusions. Wind contamination may also be considered to have contributed to these results, especially since greenhouse experiments indicate<sup>2</sup> that the interaction of the plant and the bacteria is necessary for phage production, and scrupulous care was always taken to prevent development of legumes in these boxes.

It is quite obvious that *Rhizobium meliloti* bacteriophage is widely distributed in soils bearing alfalfa; in fact, it may be said to be present in all alfalfa fields. Possibly under certain conditions it may become sufficiently concentrated and "virulent" to destroy most of the legume organisms in the soil and thus interfere with symbiosis, as Demolon and Dunez (1, 2) strongly contend. On the other hand, its presence may be as "normal" a condition as the exist-

<sup>2</sup> Unpublished results.

ence of the organisms themselves in the soil. Carefully controlled and replicated greenhouse experiments with sterile and nonsterile simple and complex (sand and soil) substrates and with resistant and susceptible organisms are essential to the solution of this cogent problem.

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# CALCIUM TRANSFER FROM MINERAL TO PLANT THROUGH COLLOIDAL CLAY<sup>1</sup>

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The processes whereby components of primary minerals become available for plant nutrition have always challenged the imagination of soil workers. Recent studies show that readily available nutrient cations must be in the exchangeable or the still more available water-soluble forms. The nutrient cations contained in the crystal lattice of the primary minerals of the silt and sand fraction are so slowly available for plants, that studies of plant tissues have yielded little information on this complex problem.

A recent study (4) showed that nutrient cations would transfer from the crystal lattice to the exchange atmosphere of the colloidal clay when certain minerals came in contact with the acid colloidal clay. Whether this transfer of ions takes place rapidly enough to be of any value to a plant in the course of a single growing season, or whether this reaction is so slow as to be significant only during long periods of time is the important question. The following investigation was undertaken with the hope of throwing some light on the very complex problem of the transfer of a nutritive cation from the crystal of a primary mineral to the plant tissue by way of the colloidal exchange surface.

## EXPERIMENTAL PROCEDURE

A pure sample of anorthite was ground very finely in a ball mill and placed in a liter cylinder, distilled water was added, and the fraction ranging in size from 0.05 to 0.005 mm. equivalent diameter was separated by means of the beaker method of mechanical analysis. The silt fraction so obtained was then placed in a Büchner funnel, and several liters of 0.001 *N* HCl were allowed to leach slowly through it. The acid treatment was followed by a complete washing with distilled water. Tests for calcium and chlorides were made to insure that all the basic cations released by grinding had been removed and that the sample was free of acid. The sample was oven-dried and reserved for later treatment.

Colloidal clay was extracted from the heavy subsoil layer of Putnam silt loam and electrodialyzed according to the method of Bradfield (1). The clay suspension thus prepared, containing 3.99 per cent of dry material, had a

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pH of 3.60. A conductometric determination of the exchange capacity according to the carbonation method of Bradfield and Allison (2) showed this to be 68 m.e. per 100 gm. of clay.

A mixture was prepared of 631 cc. of the hydrogen-clay suspension and 50 gm. of the previously treated anorthite. This mixture was allowed to stand for 100 days, during which the pH changed from 3.60 to 5.70. The amount of breakdown of the anorthite was approximately 3.4 per cent, or enough calcium was transposed from the anorthite crystals to equal about 250 mgm. or 12.5 m.e. of adsorbed Ca. To this suspension were added 4 m.e. of K as  $K_2HPO_4$ .

TABLE 1  
*Calcium uptake by soybeans*  
Data for 50 plants

CULTURE NUMBER	TREATMENT	CROP WEIGHT	CALCIUM UPTAKE		
			Proportion of crop weight	Total	Excess over seed*
		gm.	per cent	mgm.	mgm.
1	No calcium	8.69	.29	25.2	16.3
2	Calcium as anorthite	13.54	.38	51.5	42.6
3	Calcium as anorthite plus acid clay	18.97	.64	121.3	112.4

\* Fifty soybean seeds contain 8.9 mgm. of calcium.

TABLE 2  
*Composition of soybean plants as influenced by calcium in different forms*

CULTURE NUMBER	NITROGEN		MAGNESIUM		POTASSIUM		PHOSPHORUS	
	Proportion of crop weight	Total	Proportion of crop weight	Total	Proportion of crop weight	Total	Proportion of crop weight	Total
	per cent	mgm.	per cent	mgm.	per cent	mgm.	per cent	mgm.
1	2.0	174	.26	22.6	1.62	141	1.08	94
2	1.6	217	.47	63.7	1.09	147	0.62	84
3	1.3	246	.33	62.6	1.12	212	0.50	95

and 4 m.e. of Mg as  $MgSO_4$  in order to furnish other essential plant nutrients. The pH of the final mixture was 5.65, which indicates a calcium saturation of slightly more than 50 per cent. Then 2000 gm. of quartz sand that had been previously leached with 1:1 HCl for 48 hours and washed free of chlorides was added to the suspension. The sand-colloidal-clay-anorthite mixture was allowed to evaporate to optimum moisture content.

As a blank determination on the effect of the acid colloidal clay in making calcium available to the plant, a mixture was prepared as was the foregoing except that distilled water was substituted for the clay suspension and, following the mixing with sand, 1 per cent of activated charcoal was added to insure

a buffered substrate for plant growth. While standing, the anorthite-water mixture changed in pH from 5.8 to 6.2, indicating a very slight hydrolysis of calcium.

As a blank determination on the amount of calcium available in the charcoal, a substrate similar to the foregoing one but without the anorthite-water mixture was prepared.

Selected soybean seeds were germinated. After they had produced a radicle about 2 cm. long, they were inoculated, and 50 seedlings were planted in each of the three mixtures and allowed to grow for 6 weeks. At the end of this time, the tops and roots were harvested, placed in paper bags, and dried in the oven at 60°C. for 48 hours.

The analytical data represent the analyses of both the tops and the roots from the 50-plant cultures, each grown on a different substrate. The analyses for calcium, magnesium, potassium, and phosphorus were made on aliquots of a nitric and perchloric acid digestion of the pulverized plants. This digestion converted the elements into chlorides, for which the standard analytic methods were used. The nitrogen analyses were made according to the standard Kjeldahl procedure for total nitrogen.

#### RESULTS

Plate 1 shows the growth of the plants at the end of the 6 weeks. The amount of calcium transferred to the plant is evident from the data in table 1. The analyses of the digested plant material showed the content of tissue calcium to be the highest in the plants grown in culture 3 on the acid-clay-anorthite mixture. The 50 plants in this case contained 112.4 mgm. of calcium taken from the substrate. The soybean plants in culture 2 in contact with the finely ground anorthite contained only 42.6 mgm. of calcium taken from the substrate, while the plants on the charcoal, culture 1, contained only 16.3 mgm. Culture 3 contained more nitrogen and potassium than did either of the other two, as given in table 2. The amount of magnesium found was about the same in cultures 2 and 3, but was much lower in culture 1. The phosphorus content of the plants was found to be almost constant in the three cultures.

#### DISCUSSION

The results of this study show the marked effect of the colloidal H-clay in transforming a slowly available nutrient ion to a readily available one. The crystal calcium of the anorthite was not completely invulnerable to the action of the plant roots but was decidedly less available than the calcium sorbed on colloidal clay. Enough calcium was made available by root contact with the crystal to show beneficial effects in both the growth and the calcium content of the soybeans; but, by the same token, the calcium in the acid-clay-anorthite mixture was much more available to the plant.

The question naturally arises whether, in the short interval of 100 days, enough calcium would be transposed from the anorthite to increase the degree

of saturation of clay sufficiently that the calcium would be available to the plants in significant quantities for effective growth and nitrogen fixation (5). The pH of the acid-clay-anorthite mixture changed from 3.60 to 5.70, which indicated that the percentage saturation had changed from 0 to 56. This degree of saturation was high enough to produce a soybean plant containing 0.64 per cent calcium. This figure is 0.19 per cent higher than the lower limit of 0.45 per cent calcium in soybean tissue, which was shown in a previous study (3) to be necessary for the plant to carry out essential physiological functions. Both the growth and the nitrogen fixation of the soybeans undoubtedly would have been improved if a longer contact interval had been allowed to increase the degree of saturation of the colloid by calcium. When one remembers that the colloid was only approximately 50 per cent saturated, the growth of the soybeans on this substrate was excellent.

The plant analyses show that the soybeans grown on the acid clay anorthite mixture took up 112.4 mgm. of calcium, or 5.6 m.e., which is 44.8 per cent of the exchangeable calcium present at the beginning of the experiment. If this had been removed without replacement it would have reduced the degree of saturation to the low level of approximately 25 per cent. It is rather improbable that the plant would ever reduce the saturation to such a low degree, since the H ions of the clay would be continually removing calcium from the crystal lattice of the anorthite, thus keeping the calcium saturation of the clay near the initial level. Since in the substrate a source of crystal calcium was present which could maintain the base saturation, the level of exchangeable calcium on the clay was high enough to furnish the needs of the plant.

From the data and the conditions of this experiment, one might infer that a colloidal acid of some type was necessary for complete and rapid availability of plant nutrients in the soil. The process by which nonavailable nutritive cations become available might be visualized as a transfer, from the finer primary mineral crystals to a colloidal surface, of the cations, which are then picked up by the contact exchange from the colloid by the plant. Since the plant root hair, by contact with the crystal, can take nutrient cations from the primary minerals, one would conclude that the surface of the root hair maintains an interface of hydrogen ions, which act very similarly to the colloidal acid only to a much lesser degree because of lesser total surface contact. In fact, by comparing the action of colloidal acids on primary minerals with the action of plant roots, one might even approximate the amount of sorbed hydrogen maintained during a growing season at the root interface.

As a result of the data in this experiment, a procedure for a biological assay of soil mineral nutrients in the sand and silts might be suggested. The organic and clay fraction could be removed from the soil, the silt and sand washed completely and then treated with a stable electrodyalized colloid clay. The mixture should stand for several months then be added to acid-leached quartz sand. This would provide a substrate on which plants could be grown. An analysis of the nutrients in the plant tissue would serve as a test of transfer.

Further studies only can give accurate measurements on this approach to the plant nutrients held in the soil.

#### SUMMARY AND CONCLUSIONS

This study on the transfer of calcium from mineral to plant through colloidal clay gave the following results:

Plants grown on a substrate containing calcium in the crystal form as anorthite took up 42.6 mgm. of calcium.

Plants grown on a substrate in which the calcium from the anorthite had been transferred to the exchangeable form by colloidal clay took up 112.4 mgm. of calcium.

The total weight of 50 oven-dry plants grown on the culture using anorthite alone was 13.54 gm. as compared to 18.97 gm. on the culture which contained colloidal clay. Colloidal clay, therefore, becomes an effective agent in transforming slowly available plant nutrients to readily available nutrients, and should be added to the list of other better established agencies.

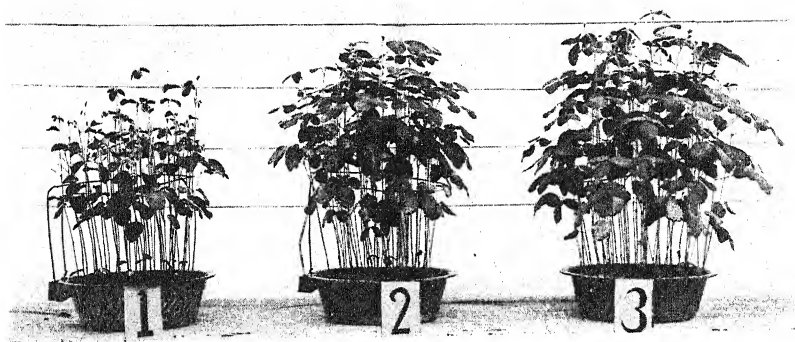
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## PLATE 1

SOYBEAN PLANTS AS INFLUENCED BY DIFFERENT FORMS OF CALCIUM

1, No calcium; 2, Calcium as anorthite; 3, Anorthite plus hydrogen clay







# INFLUENCE OF LIMESTONE AND DOLOMITE UPON SULFATE RETENTION FROM ANNUAL ADDITIONS OF POTASSIUM SULFATE

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One result of the trend toward concentrated fertilizers has been a revival of the problem of the nutrient value of sulfates for crops in humid regions. It is contended that the substantial proportion of calcium sulfate in ordinary superphosphate is of value as a source of nutrient sulfur and calcium. Since "triple" superphosphate contains a relatively meager proportion of that sulfate, the question has arisen as to whether the concentrated superphosphate is as effective as a  $P_2O_5$ -equivalence of ordinary superphosphate. Any difference in effectiveness of the two types of superphosphates is resolved into the possible benefit of sulfate sulfur, however, when needful liming is provided.

The extent to which a soil can retain sulfates against leaching will govern the length of the period during which sulfur supplied through fertilizers will be of nutritive value to shallow-rooted crops. It is important to determine whether the sulfate ion so supplied is retained by the soil sufficiently long for beneficial effect upon succeeding crops. It is important to ascertain also the effect of rational liming upon such retention.

## SOIL SULFATES

### *Derivation*

Sulfur reserves in humid soils occur chiefly as organic residues, from which sulfates are generated. Sulfur does not remain in unoxidized inorganic forms in cultivated well-drained soils of mineral origin. Sulfonation of organics and oxidation of additions of elemental sulfur and of pyrite are stimulated and sulfate outgo is accelerated by moderate supplements of all liming materials, particularly magnesian compounds at heavy rates (2, 8, 11, 12, 14, 15). Oxidation of elemental sulfur may be of either chemical or biochemical nature (5, 9, 13).

Sulfates may be brought back to surface horizons through plant roots, and variable increments of sulfates are derived from the atmosphere. The sulfur content of rainwaters, collected at ten points in Tennessee and analyzed quarterly over a 10-year period, was accounted for as sulfates of calcium, magnesium, and potassium, in that order (7). Sulfates in rainwaters are attributable

chiefly to smoke solids and therefore are governed by proximity of the collection points to urban and manufacturing centers. It has been pointed out that rainfall increments may offset rainwater leachings of sulfates from fallow soils (15). Alway, Marsh, and Methley have shown that the soil also may derive sulfur dioxide from the atmosphere, incidence of the gas being greater in urban than in rural sectors (1).

### *Losses*

Since desulfification is induced by anaerobic organisms, the sulfur content of cultivated soils presumably is depleted only through removal of crops and by the leaching action of rainwaters. Analyses of the soils from the Jordan Fertility Plots indicated that long-continued applications of ammonium sulfate caused a marked depletion of bases without build-up of sulfate content (3). Lysimeter studies have shown that outgo of both applied and soil-generated sulfates from a fallow humid soil is influenced to a variable extent by additions of calcic and magnesian materials in various forms and at different rates (4, 9). Each magnesian compound was more accelerative than its corresponding calcic compound in causing sulfate leachings, and the accelerative effect of dolomite was usually somewhat greater than that of high-calcic limestone (4, 8). A 7-year lysimeter study at the Tennessee Station dealt with the influence of cation combination upon leachability of the sulfate radical from annual equivalent additions of calcium, magnesium, and potassium sulfates, with and without single initial incorporations of limestone and of dolomite. Variation in cation combination did not affect materially the extent of sulfate outgo in the drainage waters. Absolute recoveries of the sulfate ion were obtained, however, only in leachings from magnesium sulfate and potassium sulfate treatments that had been supplemented with full-depth incorporations of dolomite (16). Results from the Virginia and Tennessee Stations (2, 4), and also unpublished findings from the Tennessee Station, showed that certain heavy subsoils effected substantial and protracted retention of the sulfate ion supplied by direct applications and also by rainwater leachings from overlying surface soil. The disparity between rates of sulfate leachings from the surface soil alone and those from soil underlain with subsoil is, however, a problem separate from the present consideration.

## EXPERIMENTAL

### *Objectives*

The objectives of the present study were to determine the influence of limestone and dolomite upon retention of the sulfate ion supplied by successive annual applications of potassium sulfate to soils exposed to rainfall and thereby to ascertain whether such treatments create sulfur reserves in the soil and how long the additions can be expected to supply nutrient sulfur to plant growth.

*Soils and treatment*

The soils used in the present lysimeter experiment were an alkaline Calhoun silt loam from the West Tennessee Experiment Station, an acidic Hartsells fine sandy loam from the Cumberland Plateau, and an acidic Cumberland clay loam from Knox County, East Tennessee. The soil area per tank was approximately 1/20,000 acre; the depth was 7 inches; there was no underlayer of subsoil; and the soils were uncropped and uncultivated during the 10-year experimental period. Initial sulfur content and water-extractable sulfates; exchange capacity; exchangeable Ca, Mg, K, and H; and pH values by glass electrode are given in table 1. The initial quantities of water-extractable sulfates in the three soils, in the order given, were 80, 100, and 220 pounds of  $\text{SO}_3$  per 2,000,000 pounds of soil. These occurrences represented 12.7, 11.1,

TABLE 1  
*Analytical values for soils of present experiment*

LABORATORY NUMBER	SOIL TYPE	pH*	EXCHANGEABLE CAPACITY†	Ca	Mg	K‡	H§	TOTAL SULFUR AS $\text{SO}_3$	WATER-EXTRACT- ABLE $\text{SO}_3$
			m.e.	m.e.	m.e.	m.e.	m.e.	per cent	per cent
6347	Calhoun silt loam	7.40	5.2	6.7	0.6	0.13	0	0.0316	0.004
6348	Hartsells fine sandy loam	5.07	9.5	1.7	0.9	0.37	7.4	0.0449	0.005
6349	Cumberland clay loam	5.61	7.9	2.6	0.4	0.35	5.6	0.0751	0.011

\* By glass electrode.

† By ammonium acetate.

‡ Procedure of Wilcox (17).

§ By difference between exchange capacity and alkalinity value.

|| Per 100 gm. soil.

and 14.6 per cent of the respective initial sulfur contents of the Calhoun, Hartsells, and Cumberland soils.

Each soil received 10 annual additions of potassium sulfate, applied in solution to the soil surface. The initial additions were made at the rate of 370 pounds per acre surface to supply 170 pounds of  $\text{SO}_3$ , with and without full-depth incorporations of 100-mesh limestone, or of dolomite, at the equivalent rate of 1 ton of  $\text{CaO}$  per 2,000,000 pounds of soil, moisture-free basis. The nine subsequent additions supplied  $\text{SO}_3$  at the rate of 178.9 pounds per annum, and the aggregate of the  $\text{SO}_3$  additions for the 10-year period was 1780 pounds, acre basis. In a fourth series, the Cumberland clay loam received fourfold annual applications of potassium sulfate, with and without single incorporations of limestone and dolomite. The fourfold additions were discontinued after the eighth treatment, however, since the composition of drain-

TABLE 2

Outgo and recovery of sulfates from ten annual additions of potassium sulfate to three Tennessee soils, with and without single initial incorporations of limestone and of dolomite

On the basis of 2,000,000 pounds of soil

SOIL		ANNUAL OUTGO OF SO <sub>4</sub> IN POUNDS										TOTAL OUTGO	RECOVERY†	
Type	Treatment*	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	lbs.	lbs.	per cent
Calhoun silt loam	None	169	93	131	98	81	63	85	90	85	100	995	....	....
	Sulfate	335	253	297	267	244	239	265	266	236	270	2672	1677	94.3
	Sulfate and limestone	350	274	302	268	242	236	277	265	245	274	2733	1738	97.6
	Sulfate and dolomite	360	273	316	288	258	247	273	278	246	275	2814	1819	102.2
Hartsells fine sandy loam	None	94	41	144	119	57	92	124	88	75	116	950	....	....
	Sulfate	192	105	405	302	187	253	325	254	217	292	2532	1582	88.9
	Sulfate and limestone	348	192	399	279	215	274	307	259	248	298	2819	1869	105.0
	Sulfate and dolomite	340	204	410	298	228	269	301	266	260	292	2868	1918	107.8
Cumberland clay loam	None	137	77	184	143	69	82	126	89	79	122	1108	....	....
	Sulfate	276	186	415	298	214	249	322	279	233	326	2798	1690	94.9
	Sulfate and limestone	480	263	386	279	209	268	281	277	244	309	2996	1888	106.1
	Sulfate and dolomite	461	274	381	290	218	265	300	282	244	282	2997	1889	106.1
	Sulfate × 4	443	703	1006	799	651	758	862	766	131	126	6245	5137	92.1
	Sulfate × 4 and limestone	941	774	899	800	731	755	751	792	94	109	6646	5538	97.3
	Sulfate × 4 and dolomite	812	741	904	793	721	820	837	838	97	118	6681	5573	98.0
Number of drainage collections per annum.....		4	3	6	7	4	4	6	5	4	6	7		
Rainfall per annum.....inches		47.0	39.5	55.3	60.8	48.0	50.7	49.5	53.4	53.9	55.6	513.7		
Rainfall increments of SO <sub>4</sub> per annum.....lbs.		86	93	95	105	103	103	115	111	114	123	1048		

\* Each initial addition applied in solution to soil surface at the equivalent rate of 170 pounds of SO<sub>4</sub> per 2,000,000 pounds of soil, and each subsequent addition at the rate of 178.9 pounds, representing a total of 1780 pounds. Single initial incorporations of limestone and dolomite at rate of 1 ton CaO per 2,000,000 pounds of soil.

† Proportion of the total addition of 1780 pounds of SO<sub>4</sub> for the 10 additions at the basal rate, and 5689 pounds for sulfate × 4 additions which were terminated after one addition at the rate of 680 pounds and seven annual additions of 715.6 pounds, when the soils had ceased to show evidence of K fixation.

age waters then showed that potassium fixation had ceased. The aggregate of the eight heavy treatments was 5689 pounds of  $\text{SO}_3$  per acre.

Annual analyses, inches of yearly rainfall and sulfate increments therefrom, totals of sulfate additions, and percentage recoveries, are given in terms of  $\text{SO}_3$  in table 2. The potassium sulfate treatments of the present comparisons were made in parallel with equivalent additions of other potassic salts, and the annual composites of periodic rainwater leachings therefore were analyzed for K, Ca, and Mg, as well as sulfates. The present paper, however, deals solely with the fate of the added sulfate radical.

#### EXPERIMENTAL FINDINGS

The difference between sulfate outgo from untreated soil and sulfate input by rainwaters affords a measure of the inherent tendency of the soil to retain sulfates. Sulfate outgo from the untreated Hartsells soil during the initial annual period was only slightly more than increments by rainwater, whereas the sulfate outgo from each of the other two untreated soils was in substantial excess of the sulfates derived from the rainwater of that year. The sulfate outgo (169 pounds) from the untreated Calhoun soil during the first year was almost identical to the sum (166 pounds) of its initial content of water-extractable sulfates and increments from rainwaters. Sulfate removal by the leachings from the untreated Hartsells soil during the first year was about half of the sum of initial sulfate content and sulfate increments. The same was true for sulfate release from the untreated Cumberland clay loam. In some cases, subsequent annual outgo of sulfates from the untreated controls was considerably less, and in others somewhat greater, than the amount of sulfates derived from the rainwaters. The 10-year aggregate of sulfate increments from rainwaters exceeded the respective amounts that passed through the unsulfated Calhoun and Hartsells soils. These two soils were comparable as to their initial contents of total sulfur and water-extractable sulfates. The high initial water-soluble sulfate content, 220 pounds of  $\text{SO}_3$ , of the Cumberland soil was reflected by an aggregate outgo of 60 pounds of  $\text{SO}_3$  in excess of the quantity brought to it by rainfall.

#### *Sulfate recoveries*

*From unlimed soils.* The sulfate outgo from each unsulfated soil was used as the control in computations as to recovery of the sulfate additions. The difference between the *initial* annual sulfate outgo from each *unlimed* sulfated soil and the first year's outgo from its untreated control was less than the sulfate addition. Recovery from the Hartsells soil for the first two years was less than half of the addition, as against recoveries of 71 per cent and 94 per cent from the unlimed Cumberland and Calhoun soils. During succeeding years, enhancement in outgo of sulfates for an annual period was less in some cases and in other slightly more than the equivalent of the annual addition of  $\text{K}_2\text{SO}_4$ . The cumulative outgo of sulfates from each unlimed sulfated soil

proximated but in most cases did not equal the cumulative additions during the interval between the first and tenth annual periods.

Some retention of sulfates was indicated by the final results from each unlimed soil. Minimal percentage recovery, or maximal percentage retention, for the 10-year period was registered by the Hartsells soil in its natural acidic state. The largest quantity of sulfates not accounted for by the leachings was the fourfold addition to the unlimed Cumberland soil. Comparison of initial total sulfur content and that found by fusion of the final sample did not demonstrate that the fourfold treatment had accumulated sulfur in forms other than sulfates. The possibility that this apparent discrepancy was due to desulfonation is being considered in supplemental studies.

An attempt to correlate sulfate outgo and total rainfall for a particular annual period involves detailed considerations. Extent of rainwater leaching of soluble salts during a given period is governed probably more by distribution, or periodicity, of rainfall than by the total precipitation. When precipitation during the latter part of an annual period is subnormal, sulfates accumulate and are removed to considerable extent by the rainfall of the early period of the succeeding year.

*From limed soils.* Enhancements in sulfate outgo from the limed soils during the first year represented complete recoveries of the initial sulfate additions. The accelerative influence of the liming materials upon sulfate outgo was still in effect during the second annual period but not definitely thereafter. The respective increases in sulfate outgo from the limed Hartsells and Cumberland soils during the first two years were respectively equivalent to one sixth and one half of the sulfate additions.

At the end of the 10-year period both limestones had induced substantially absolute sulfate recoveries from all of the additions at the basal rate, and in five of the six cases there was evidence of a draft upon the sulfates derived jointly from the soil and its rainwater increments. The effect of dolomite was somewhat greater than that of high calcic limestone upon outgo of sulfates from the Calhoun and Hartsells soils. The dolomite contained small quantities of sulfides of iron and zinc, the complete oxidation of which would have supplied 55 pounds of  $\text{SO}_2$  per acre. Limestone and dolomite were equally effective, however, in the Cumberland clay loam and were of almost identical effect upon 8-year and 10-year recoveries from the 5689-pound aggregate of the fourfold additions.

The aeration incident to the incorporation of the liming materials was imposed also upon the unlimed soils and of itself was conducive to increased sulfonation. In previous related studies, it was observed that outgo of biological end-products from untreated soils was maximal during the first year after placement. The incorporation of ordinary liming materials at economic rates usually induces further enhancement in outgo of solutes during the first year, and even during the second year. The greater recovery of sulfates from the limed soils during the first two years probably can not be attributed

altogether to activated sulfonation, since the acidic soils retained substantial fractions of the sulfates added during the first two years. The ameliorative effect of the liming materials upon the colloids of the amphoteric elements apparently diminished their capacity to induce the formation of insoluble hydroxy sulfate complexes.

TABLE 3

*Correction of sulfate-leaching recoveries by application of differences between initial and final amounts of water-extractable sulfates\**

Sulfate in pounds of  $\text{SO}_4$  per 2,000,000 pounds of soil

SOIL			SULFATE OUTGO, DEVIATION FROM UNTREATED SOIL		FINAL WATER- EXTRACTABLE SULFATE CONTENT		COMPUTED RETEN- TION, $-\frac{\dagger}{\dagger}$ , AND RELEASE, $+\frac{\S}{\S}$ , OF SULFATE BY SOIL
Type	Treatment	Final pH values $\dagger$	Actual	In re- lation to re- covery	Actual	Devi- ation from initial content	
Calhoun silt loam	Sulfate	6.97	1677	-103	68	-12	-115
	Sulfate and limestone	7.60	1738	-42	88	+8	-34
	Sulfate and dolomite	7.50	1819	+39	98	+18	+57
Hartsells fine sandy loam	Sulfate	5.00	1582	-198	128	+28	-170
	Sulfate and limestone	5.70	1869	+89	90	-10	+79
	Sulfate and dolomite	5.70	1918	+138	90	-10	+128
Cumberland clay loam	Sulfate	5.20	1690	-90	150	-70	-160
	Sulfate and limestone	6.00	1888	+108	110	-110	-2
	Sulfate and dolomite	6.10	1889	+109	110	-110	-1
	Sulfate $\times$ 4	5.30	5137	-552	116	-104	-656
	Sulfate $\times$ 4 and limestone	6.10	5538	-151	92	-128	-279
	Sulfate $\times$ 4 and dolomite	6.10	5573	-116	88	-132	-248

\* Fifteen minutes' agitation of 100 gm. of soil per 500 cc. of water; 400 cc. of Büchner filtrates evaporated with  $\text{Mg}(\text{NO}_3)_2$ ; residue ignited, taken up with dilute  $\text{HCl}$ ; silica removed and sulfates precipitated as  $\text{BaSO}_4$ .

$\dagger$  All pH values by glass electrode. Initial and final pH values of untreated soils were: Calhoun, 7.40 and 7.00; Hartsells, 5.07 and 5.00; Cumberland, 5.61 and 5.10.

$\ddagger$  Upon assumption that no loss can be attributed to gaseous phases of S compounds.

$\S$  Algebraic sum of actual excess in outgo and variation between initial and final content of water-extractable sulfates.

#### *Verification of lysimeter findings as to sulfate recoveries*

The sulfate recoveries of table 2 were based upon assumptions that the amount of leachable sulfates in each soil at the end of 10 years was the same as the amount present initially and that the sulfates of the fallow soils were not deoxidized into gaseous phase and not transformed into organic combinations. The two last-mentioned assumptions were deemed tenable, but it was considered probable that initial and final sulfate contents were different



because of the physical and chemical changes induced in the soils by the several treatments.

Differences between lysimeter recoveries and those corrected by applying the variations between initial and final quantities of water-extractable sulfates are given in the last column of table 3. Considered as ultimate effects of ten additions during the 10-year period, the recovery values registered by the lysimeter data are substantially the same as the values obtained by application of variations between initial and final contents of water-extractable sulfates.

*Relation of soil reaction to sulfate leachability*

Changes in pH values of the lysimeter soils are given in table 3 and show that the natural rainwater leaching invariably caused a decrease in pH of

TABLE 4

*Disparity between amounts of residual sulfates removed by agitated aqueous and dilute hydrochloric acid extractions of soils after 10 annual additions of potassium sulfate\**

Sulfate in pounds of  $\text{SO}_3$  per 2,000,000 pounds of soil

SOIL			SULFATE EXTRACTIONS		
Type	Treatment	pH†	By water	By 0.05 N HCl	Disparity by acid extraction
Calhoun silt loam	Sulfate	6.97	68	22	46
	Sulfate and limestone	7.60	88	26	62
Hartsells fine sandy loam	Sulfate	5.00	128	20	108
	Sulfate and limestone	5.70	90	18	72
Cumberland clay loam	Sulfate	5.20	150	20	130
	Sulfate and limestone	6.00	110	16	94
	Sulfate $\times$ 4	5.30	116	10	106
	Sulfate $\times$ 4 and limestone	6.10	92	12	80

\* Technic identical with that described in corresponding footnote of table 3.

† By glass electrode.

each untreated soil. The same was true of the soils that received potassium sulfate alone, although the sulfate additions apparently exerted no effect upon pH. The 1-ton  $\text{CaO}$ -equivalent liming treatments maintained the initial alkalinity of the Calhoun soil, but were not sufficient to impart ultimate alkalinity to the two initially acidic soils. The two types of limestone were equally efficacious in their effect upon final pH.

The leaching data suggested the conclusion that diminution in acidity decreases the capacity of a soil to retain added soluble sulfates. The influence of pH upon the capacity of each of the three soils to retain sulfates was studied through simultaneous aqueous and 0.05 N HCl extractions of samples of certain of the lysimeter soils collected at the end of the 10-year period, by the

analytical technic indicated in the first footnote of table 3. The data of table 4 show that sulfate removal by means of the agitated acidic extraction of each soil was only a small fraction of the removal effected by agitated aqueous extraction. This held for the sulfated Calhoun soil, both limed and unlimed. That soil was alkaline initially and practically neutral at the end of the 10-year period, whereas definite alkalinity had been maintained by the liming. The dilute acid converted the analytical charge of limed Calhoun soil to an acidic system and extracted almost the same amount of sulfates from it and the unlimed initially alkaline soil. Attempts to effect recovery of added sulfates by means both of fusions and of incinerations of sulfate-fortified soils demonstrated that recoveries by aqueous extractions exceeded those obtained by similar extractions with mineral acids (6).

Each addition of limestone and of dolomite had induced a tendency toward sulfate release to rainwater leachings, although none of the six liming treatments induced a final pH above 6.10 in the Hartsells and Cumberland soils. The tendency of the partially neutralized acidic soils of the last three groups of table 3 to yield larger quantities of sulfates to rainwater leachings, and also to aqueous extractions, was nullified by the substantial acidity imparted to the charges of soil by the dilute acid extractant.

This marked alteration in the capacity of both unlimed and limed soils to retain sulfates, subsequent to lowering the pH in dilute acid suspensions, serves to substantiate the explanation as to disparities in sulfate recoveries by the rainwater leachings from identical sulfate additions to the several limed and unlimed lysimeter soils. Incomplete recoveries of sulfate additions and rainwater sulfates by the rainwater leachings from the acidic soils are probably due to development of retentive capacities, rather than either to chemical reduction and dissipation of the added sulfates or to the formation of organic compounds. The recoveries of sulfates beyond the quantities introduced into the limed soils are attributable in part to increased sulfonation, particularly during the early years of the 10-year period, and in part to the effect of liming upon sulfate leachability, which is also a measure of the capacity of the soil to supply solute sulfates to plants.

#### PRACTICAL ADAPTATION OF FINDINGS

The leaching data demonstrate that no substantial build-up of the sulfur content of the soil is to be anticipated from the addition of potassium sulfate at rational rates. Since the sulfate retention from additions of this sulfate have been shown to be practically identical to retentions from equivalent additions of both calcium and magnesium sulfates (16), it seems admissible to translate the present findings into terms of calcium sulfate derived from additions of superphosphate.

Annual incorporations of approximately 675 pounds of ordinary superphosphate of 45 per cent calcium sulfate content would be required to furnish the 178-pound yearly additions of  $\text{SO}_3$  supplied by the potassium sulfate treat-

ments. The sulfate leachings from the fallow soils indicate that the acidic soils retained substantial fractions of sulfate from additions made at that rate. Such additions would be leached within the year from moderately limed soils of the types used in this experiment.

Although rational additions of ordinary superphosphate to general crops seldom supply more than 178 pounds of  $\text{SO}_3$ , it was considered desirable to apply sulfate-sulfur at a rate beyond one to be expected in fertilization for special "money" crops. The behavior of the fourfold incorporations was similar to that of the incorporations at the basal experimental rate, and there was no justification for the expectation of a substantial build-up of sulfur in the moderately limed soils.

Until evidence to the contrary is adduced, it can be postulated that the principles that govern the fate of sulfate supplied by superphosphate treatments in the range of 675 to 2700 pounds per acre per annum are applicable also for smaller incorporations, at least in the case of Cumberland clay loam.

There would be no point to an assumption that the retention of sulfates by the unlimed soils would represent an advantageous accumulation of sulfur reserves. Rational usage of acidic phosphates calls for supplemental liming on most of the soils of humid regions, and nothing would be gained by an accumulation of sulfates in unlimed soils that would show no economic crop response to acidic phosphates. It seems logical to conclude that sulfates supplied to limed soils through additions of fertilizers will afford current supplies of readily available nutrient sulfur and that such supplies are removed within a year and hence do not accumulate in soils of humid sectors. Moreover, rational liming would supply adequate quantities of nutrient calcium, and this would minimize the beneficial effect of the supply of nutrient calcium introduced by the calcium sulfate of ordinary superphosphate. Any benefit from the calcium sulfate content of superphosphate additions to limed soils would be attributable therefore to the nutritive value of sulfate sulfur.

#### SUMMARY

One alkaline soil and two acidic soils were used in a 10-year lysimeter study of the fate of the sulfate ion from annual applications of  $\text{K}_2\text{SO}_4$  to fallow surface, with and without initial full-depth incorporation of a 1-ton  $\text{CaO}$ -equivalence of either limestone or dolomite.

Sulfate leachings from the untreated soils were less than sulfate increments from rainwaters for some annual periods and greater for others. The 10-year aggregates of sulfate leachings from two of the untreated soils were less than the total amount of sulfates introduced by rainwaters, whereas the reverse was true of the third untreated soil.

Absolute recovery of sulfate additions for the 10-year period was not obtained from any of the unlimed soils, the recoveries being in the percentage range of 89 to 95.

The early effect of the single incorporations of each liming material was

to accelerate sulfate outgo. The ultimate effect was complete recovery of the added sulfate radical and a draft upon the sulfur content of the soil and its rainwater increments.

Correction of aggregate sulfate outgo by application of the appropriate algebraic difference between initial and final contents of water-extractable sulfates did not alter appreciably the lysimeter findings as to sulfate recoveries.

The rainwater leachings induced a lowering of pH in every soil, but the sulfate additions, *per se*, exerted no effect upon pH values. A lowering of pH imparted a marked increase in the capacity of each soil to retain sulfates.

Evidence of the tendency of additions of sulfates to enter into less readily leached sulfate complexes in the acidic soils was adduced by disparities between the amounts of sulfates recovered from the final samples of the lysimeter soils by simultaneous aqueous and 0.05 N HCl extractions. Each sulfate removal by aqueous extraction was much greater than the removal by dilute acid extraction.

#### CONCLUSIONS

Since a previous study showed substantially identical leachability of the sulfate radical in its combination with calcium, magnesium, and potassium, it seems allowable to interpret the present results in terms of sulfate retention when the soil receives economic additions of ordinary superphosphate.

The results indicate that the rainfall of humid regions effects substantially complete removal of ordinary additions of fertilizer sulfates from the soil within a year. Acidic soils may retain small fractions of the sulfate additions, but supplemental incorporations of finely ground limestone and dolomite accelerate and enhance the outgo of sulfates to an extent that precludes a build-up of sulfur from the sulfate applications. Successive annual additions of potassium sulfate exert no direct influence upon the ultimate pH of the soil.

The findings indicate that the directly beneficial effects attributable to nutrient sulfur supplied by the sulfate components of ordinary additions of fertilizers would not extend to crops grown in succession to those fertilized.

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# NITROGEN FIXATION BY AZOTOBACTER CHROOCOCCUM IN THE PRESENCE OF SOIL PROTOZOA<sup>1</sup>

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That protozoa occur in soil has been recognized since the work of Ehrenberg a century ago, but it was not until 1909 that Russell and Hutchinson (35) advanced the theory that protozoa limit bacterial numbers. Previous to this investigation, protozoa were believed to be accidental contaminants of the soil, occurring only as passive cysts. With the recognition that protozoa are active agents in soil processes, research in the field of bacterial-protozoan relationship was actively pursued in both Europe and America (1, 6, 8, 14, 36, 38, 39, 42, 45), and today many soil microbiologists believe protozoa increase beneficial bacterial activities.

Sewertzova (41) found that protozoa prefer *Azotobacter* to many other soil bacteria, and Fedorowa-Winogradowa (12) cultured amoebae on *Azotobacter* as the sole source of food.

Kopeloff, Lint, and Coleman refer to several investigations reporting the abundance of protozoa in crude cultures of *Azotobacter*. Baumgärtel (2) noticed that protozoa were abundant in *Azotobacter* cultures and that the pellicle was modified depending upon the type of available nitrogen in the soil. Nasir (29) and Cutler and Bal (9) found that several kinds of soil protozoa stimulated nitrogen fixation by *Azotobacter*. This was confirmed with amoebae by Fedorowa-Winogradowa (13). Hirai and Hino (21), working with *Azotobacter* and protozoa in sand cultures, reported stimulation of nitrogen fixation in a majority of cases owing, they believed, to a disjunctive symbiosis between the two organisms. Telegdy-Kovats (43) found that Colpoda and Paramecium stimulated *Azotobacter*. Lander (25) reported that protozoa increased nitrogen fixation 48 per cent. Moler (28) assumed that protozoa make the nitrogen in *Azotobacter* cells available to plants, the assimilated bacterial protein being excreted as urea, ammonia (11), or uric acid (22).

## METHODS OF INVESTIGATION

### *How cultured*

The methods used to study the relationship of protozoa to *Azotobacter chroococcum* are similar to those employed by previous workers. The protozoa

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in all cases were freed from bacteria, the *Azotobacter* serving as the sole source of nutrient. Instead, however, of using one type of substrate such as liquid medium or soil in which to study the effect protozoa exert on nitrogen fixation, four different substrates—liquid, agar, sand, and soil media—were used. The liquid and agar media were prepared from pure chemicals and distilled water. In the earlier experiments a modified Greaves' medium (16) containing 5 p.p.m. of iron and manganese was used. In a few experiments, 1 p.p.m. of sodium molybdate was added to the liquid medium. Sterilized soil was the third medium. In it a study was made of the influence of moisture, the influence of humus, and the influence of mannite.

The liquid medium was distributed in 100-cc. portions into 1000-cc. Erlenmeyer flasks. Twenty-four cubic centimeters of the agar was poured into large Petri dishes. The sand and soil substrates were distributed in 100-gm. quantities in large Petri dishes and in covered tumblers. The soils varied in humus content, and they were autoclaved for 7 hours at 117°C. Tests showed them to be sterile. To each sand container was added aseptically sufficient nitrogen-free mineral solution to saturate the sand. To part of the soil containers was added the equivalent of 1 gm. of mannite. Several different moisture contents were studied.

#### *Protozoan cultures*

The protozoan species were obtained from five different soils; four from the Utah Experiment Station Greenville farm, which had received different manure or fertilizer treatment over a period of years, and the fifth, rich in leaf mold, from a forested area near Logan, Utah.

Forty-gram portions of the soils were placed into 50 cc. of each of the 14 different media in 250-cc. Erlenmeyer flasks, according to Yakimoff and Zérèn (46). After a few days the inoculated flasks were examined. The maximum number of protozoan species found was ten; of these, six species commonly occurred. Four protozoan species were used in this work: two ciliates, *Colpoda maupasii* and *Oxytricha* sp.?; one amoeba, *Hartmanella hyalina*; and one flagellate, *Colponema symmetrica*. They were isolated by means of capillary pipettes. Depression slides were used as dilution chambers. The process of dilution and examination continued until only one protozoan cell remained in a drop. This was incubated in a moist chamber for 24 hours and, if found free of other protozoa, was transferred to fresh liquid medium.

The amoebae obtained in "pure mixed cultures" (a pure culture of protozoa growing on a pure culture of bacteria) were purified by the Petri-dish migration methods of Sewertzova (40) and Oehler (30, 31). The ciliate *Colpoda* was freed of the majority of the contaminating bacteria by the micro-pipette-washing technic of Hargitt and Fray (19) and Parpart (32), after which a pure mixed culture was obtained as described previously for the amoeba. A method described by Hall (18, pp. 51-59), in which advantage is taken of the geotropic responses of these organisms, was used successfully with both the ciliates and with the flagellate.

*Biological determinations*

Two days in advance of the protozoan inoculation, the various media were inoculated with known quantities of *Azotobacter* cells, thus ensuring a substrate immediately suitable for the growth of the trophic protozoa. The same numbers of protozoan cells were used to inoculate each flask.

*Bacterial numbers.* Bacterial numbers were determined by the direct microscopical method, twenty fields being counted and the average taken. Determinations were made in replicate. The liquid media flasks were made up to volume and well shaken to distribute the cells as evenly as possible. For staining, erythrosin was found to be most satisfactory, since it did not appreciably stain the capsular material accompanying the bacterial cells. The growth was carefully scraped from the surface of the agar medium and suspended in 100 cc. of sterile water, thoroughly shaken, and 1-cc. aliquots were removed and counted. In the sand cultures, 1-gm. quantities of the moist sand were vigorously shaken in 250-cc. Erlenmeyer flasks containing 9 cc. of sterile water. The organisms in the supernatant liquid were then counted. The method of Conn (5) was used on the soil counts.

*Protozoan numbers.* The protozoa in the liquid and agar media were counted according to Peters (33). The protozoa in the soil and sand cultures were counted by the dilution method. No attempt was made to distinguish between trophic and cystic forms. Dilutions up to 1-10,000 were used.

*Chemical determinations*

Nitrogen was determined by the Kjeldahl method. In these determinations, 20 gm. of the sand, 10 gm. of the soil, and the total liquid and agar cultures, allowance being made for the aliquots removed, were used. Mannite consumption was determined by the Christensen and Bondorff (4) method, the pH by a quinhydrone electrode and calomel half cell. All figures on nitrogen reported represent mean values derived from closely agreeing replicates.

## EXPERIMENTAL RESULTS

*Experiments on liquid media*

The liquid media after inoculation were incubated from 2 to 6 weeks. The first experiments lasted 4 weeks with incubation temperatures of 20 and 30°C. *Colpoda maupasii* was used as the coexistent organism. Each flask was inoculated with 5,000 *Colpoda* and 8,000,000 *Azotobacter* cells and contained 1 gm. of mannite. The results, averages of three or more closely agreeing determinations, are given in table 1.

The quantity of nitrogen fixed by *Azotobacter* alone is small. This is owing to its having been cultured for 6 months on media prepared with Baker's pure chemicals and distilled water. Moreover it is a characteristic of *Azotobacter chroococcum* to decrease in nitrogen-fixing ability when cultured in synthetic media. This culture, after being passed through sterile soil, fixed 5.2



mgm. of nitrogen. To assure uniformity, the same culture was used throughout the experiment.

The quantities of nitrogen fixed in the presence of the Colpoda are decidedly greater than the controls, and since the only difference between the two sets of cultures is the protozoan cells themselves it must be concluded that these in some way stimulate *Azotobacter*.

It is apparent from table 1 that in addition to the greater quantities of nitrogen fixed in the Colpoda-*Azotobacter* cultures there is a more rapid consumption of mannite. By the end of 29 days, however, the two cultures had consumed nearly all the mannite. Hence for energy utilized, the Colpoda-*Azotobacter* cultures fixed two to three times as much nitrogen as did *Azotobacter* alone.

TABLE 1

*Nitrogen fixed, mannite consumed, and number of Azotobacter in liquid medium in the presence and absence of Colpoda*

	AT 20°C.			AT 30°C.	
	7 days	14 days	29 days	14 days	29 days
Nitrogen fixed, mgm.					
<i>Azotobacter</i> cultures.....	0.8	1.2	2.7	1.4	3.2
<i>Azotobacter</i> -Colpoda cultures.....	5.4	9.1	9.6	6.1	6.4
Mannite used, mgm.					
<i>Azotobacter</i> cultures.....	150	730	870	660	960
<i>Azotobacter</i> -Colpoda cultures.....	620	930	950	920	980
Number of <i>Azotobacter</i> , millions/cc.					
<i>Azotobacter</i> cultures.....	20.7	23.1	34.2	70.0	67.0
<i>Azotobacter</i> -Colpoda cultures.....	68.9	85.1	54.3	64.6	76.7

The temperature influenced the quantity of nitrogen fixed. Pure cultures of *Azotobacter* were favored by higher temperature, whereas the Colpoda-*Azotobacter* cultures did better at 20°C. As most ciliates are known to develop best at 20 to 22°C., it seems that the increased fixation at 20°C. may be due to a more favorable temperature for the protozoa, even though 30°C. is optimum for *Azotobacter*. That the protozoa developed better at the lower temperature than at the higher is evident from the protozoan count after 14 and 29 days. At 20°C. there were 25,000 and 76,000 cells per cubic centimeter as against 10,000 and 36,000 for the same periods at 30°C.

The substantially greater number of *Azotobacter* cells usually found in the Colpoda-*Azotobacter* cultures accounts for the increased nitrogen fixation and the rapid disappearance of mannite. Though the difference in bacterial numbers in the Colpoda-*Azotobacter* and control cultures is definite at 20°C., it is less so at 30°C. There were also visible differences owing to the presence

of Colpoda. The pellicles which formed after about a week in the Colpoda-Azotobacter flasks were thick and filled with minute holes, and the Colpoda organisms were found directly under the membrane, whereas the pellicles on the control cultures were thin and transparent. After about 4 weeks the pellicle on the Colpoda-Azotobacter cultures became dark brown or black and extremely thick and leathery. The bacterial cells in the Colpoda-Azotobacter cultures were morphologically different from the cells in the controls. The majority of the former were relatively small and stained deeply, as is characteristic of young cells. Granules were absent.

This experiment may be criticized because of the low fixation of nitrogen by the controls; therefore, in another test 1 p.p.m. of sodium molybdate was added to the medium previously used. The controls fixed 12.2 mgm. and the Colpoda-Azotobacter cultures 17.5 mgm., indicating stimulation by the protozoa even in the presence of molybdate. Moreover the fixations in our controls are not far different from those reported by Cutler and Bal (9).

The question arises as to whether this stimulation is temporary or whether it would continue for a longer period. To answer this, 1 per cent mannite was added to each flask every 2 weeks. By the end of 90 days an average of 32 mgm. of nitrogen had been fixed by the Colpoda-Azotobacter cultures and 8 mgm. by the control. Six grams of mannite had been added to each flask. The Colpoda-Azotobacter cultures lost little of their original efficiency, as they fixed 7 mgm. during the first 15 days and 11 mgm. during 30 days. Apparently the cultures could have gone on indefinitely fixing nitrogen, provided they had a supply of mannite and phosphate. The controls, on the other hand, fix nitrogen slowly, decomposing the mannite for purposes other than fixation. Bacterial numbers were much greater in the Colpoda-Azotobacter cultures.

Previous workers have offered several possible explanations for the increased fixation observed in the presence of protozoa. Cutler (9) and others (29, 43, 44) believe that the phagocytic activity of the protozoa maintains bacterial activity at maximum efficiency by eliminating old cells and their metabolic products, thus permitting younger cells to continue their activity at a higher rate. Hirai and Hino (21) suggested that the Azotobacter and protozoa are mutually beneficial, the bacteria by serving as food and the protozoa through excretion of ammonia, which maintains the alkalinity of the medium by neutralizing the organic acids produced.

Neither of these theories seems adequate to explain fully the data obtained by the authors. Phagocytic activity undoubtedly occurs but not to such a degree that the bacterial numbers were reduced below those of the controls. The reaction of the Colpoda-Azotobacter cultures (pH 8.17) after 4 weeks was decidedly more alkaline than that of the controls (pH 7.28) and even more alkaline than that of the uninoculated media (pH 7.85).

*Influence of pH.* The great numbers of young active cells together with the alkalinity of the media may explain the increased nitrogen fixation. To learn whether this was the case, an experiment similar to the first was tried

in which buffered and unbuffered media were used. Here *Hartmanella hyalina* and *Colpoda maupasii* were used (table 2).

The important fact in this experiment is that although the protozoa are able to render the medium alkaline in reaction they do not maintain an alkaline reaction during the first week of incubation. Yet it is during this period that fixation is most active in the protozoa-Azotobacter cultures. Hence it is evident that Hirai and Hino's explanation is not sufficient, since the optimum pH for fixation—7.5—(3) is reached subsequent to the intensive period of fixation and is not maintained. On the other hand the pH of the controls is

TABLE 2  
Numbers of protozoa and Azotobacter, pH, and nitrogen fixed in liquid medium at 24°C.  
Initial pH of unbuffered medium 7.85

	UNBUFFERED MEDIA		BUFFERED MEDIA	
	7 days	16 days	7 days	16 days
Colpoda, 1000/cc.....	27.5	35	0.2	0.36
Amoebae, 1000/cc.....	9.5	11.2	2.2	4.8
Azotobacter, millions/cc.				
Azotobacter cultures.....	31.6	27.7	49.3	49.9
Azotobacter-amoebae cultures.....	25.2	67.1	58.9	52.7
Azotobacter-Colpoda cultures.....	154.3	68.3	112.6	98.4
pH of:				
Azotobacter cultures.....	7.25	7.10	7.35	7.28
Azotobacter-amoebae cultures.....	7.03	7.23	7.03	7.07
Azotobacter-Colpoda cultures.....	6.83	7.53	6.86	7.50
Nitrogen fixed, mgm.				
Azotobacter cultures.....	1.2	2.1	1.8	3.0
Azotobacter-amoebae cultures.....	1.5	2.3	2.3	3.1
Azotobacter-Colpoda cultures.....	5.8	6.9	6.8	8.5

reduced very slowly, and if reaction were alone responsible for the fixation more nitrogen should have been fixed in these cultures.

*Influence of killed cultures.* The fact that a few hundred Colpoda cells per cubic centimeter in the buffered media were as effective in stimulating fixation as many thousands of cells in the unbuffered media indicated that phagocytic action was inadequate to explain the results and perhaps a substance necessary in only traces was responsible. To test this theory small Erlenmeyer flasks containing 25 cc. of liquid medium were inoculated with Azotobacter and incubated until a good growth had developed. A Colpoda-Azotobacter culture containing thousands of protozoan cells per cubic centimeter was heated at a temperature of 65°C. for one-half hour and allowed to stand for 24 hours, after which it was examined for living protozoa. Heating had killed both

trophic cells and cysts. Five cubic centimeters of the dead protozoan suspension was introduced into one half of the flasks; the other half were kept as controls. Other flasks received similar 5-cc. quantities of protozoan suspension and were analyzed for nitrogen, which was subtracted from the nitrogen in the treated flasks. Triplicate determinations were made at the end of 4, 9, and 20 days. At the end of the fourth and ninth days additional 5-cc. quantities of dead Colpoda suspension were added to each of the treated flasks. Careful inspection of the treated flasks showed no living Colpoda. Results are reported in table 3. The pH values of the two sets of cultures, the controls and the treated cultures, are the same; both decreased below the pH of the uninoculated medium. The introduction of the dead protozoan suspension had not prevented the pH from declining, yet it increased fixation. At the end of the fourth and ninth days the ratio of nitrogen in the treated flasks to the controls was 3 to 1. The ratio became narrower by the twentieth day, however, primarily because of the earlier exhaustion of the mannite in the treated cultures. The results indicate that *Colpoda maupasii* may produce a

TABLE 3  
*Nitrogen fixed in the presence and absence of heat-killed suspension of Colpoda*  
Initial pH 7.85

	AZOTOBACTER ALONE			AZOTOBACTER AND DEAD COLPODA SUSPENSION		
	4 days	9 days	20 days	4 days	9 days	20 days
Nitrogen fixed, mgm.....	0.4	0.6	2.1	1.3	2.0	2.6
pH of media.....	...	7.38	...	...	7.39	...

growth-promoting substance which is thermostable at 65 to 70°C. This substance may accelerate cell division, thus explaining the greater numbers of Azotobacter cells found in many of the Colpoda-Azotobacter cultures. Robertson (34) presented evidence of the production of such a growth-promoting substance by yeast, bacteria, and protozoa. Apparently mere traces of the growth-accelerating substance are effective, since the products of a few hundred protozoa per cubic centimeter in the buffered medium were as effective as several thousand cells in the unbuffered medium (table 2).

In addition to the foregoing, Seitz filtrates from living and heat-killed suspensions were used in concentrations ranging from 0.02 to 5 cc. per 100 (tables 4 and 5). The stimulating substance either is not soluble or if soluble does not readily pass through a Seitz filter, since in only one of several tests made with filtrates was any stimulation observed. It is evident from the data that the heat-killed suspensions in small concentrations (1 cc.) stimulate fixation and cell division to as great a degree as the living cells. A majority of the bacterial cells in such cultures have the characteristic appearance of young cells. The stimulating substance is probably protein, as it does not pass

through a Seitz filter. Greaves, Jones, and Anderson (17) found that nitrogen fixation is stimulated by casein, albumen, and some amino acids. The greatest stimulation occurred where casein was used, and after the casein was washed the stimulation decreased.

TABLE 4

*Nitrogen fixed and number of Azotobacter chroococcum when grown in the presence of heat-killed suspension and Seitz filtrate of Colpoda maupasii*

COLPODA SUSPEN- SION ADDED	BAC- TERIAL COUNT	NITRO- GEN FIXED	pH*	MANNITE CONSUMP.	COLPODA EXTRACT ADDED	BACTERIAL COUNT	NITRO- GEN FIXED	pH*	MANNITE CONSUMP.*
cc.	millions	mgm.		mgm.	cc.	millions	mgm.		mgm.
<i>Incubated at 30°C.</i>									
5	168	6.4	7.67	850	5	70	2.0	7.58	470
1	142	3.8	7.63	860	1	35	1.8	7.55	700
0.2	56	3.8	7.35	870	0.2	64	1.6	7.44	610
0.02	75	3.8	7.52	860	0.02	105	2.0	7.42	580
				Inoculated control	21	2.1	7.35		430
<i>Incubated at 20°C.*</i>									
5	191	5.7	7.34	860	5	48	2.4	7.36	770
1	173	3.3	7.45	870	1	30	2.3	7.37	580
0.2	143	3.7	7.30	890	0.2	42	2.4	7.10	670
0.02	106	3.4	7.27	840	0.02	42	2.2	7.17	690
				Inoculated control	62	2.5	7.21		570

\* Uninoculated control: pH 7.78, 1000 mgm. mannite.

TABLE 5

*Number of Azotobacter chroococcum and quantity of nitrogen fixed in the presence of varying quantities of heat-killed suspension and Seitz filtrate of Colpoda maupasii*

PROTOZOAN MATERIAL ADDED	NITROGEN FIXED				BAC- TERIAL COUNT AFTER 34 DAYS	pH			
	7 days	18 days	24 days	34 days		7 days	18 days	24 days	34 days
	mgm.	mgm.	mgm.	mgm.					
Inoculated control.....	1.8	1.5	1.8	2.2	26	7.35	6.00	7.30	6.95
1 cc. of filtrate.....	2.8	3.1	4.7	5.2	72	6.75	6.95	7.15	7.03
5 cc. of filtrate.....	2.3	3.3	5.3	5.9	52	7.13	7.10	7.03	7.17
1 cc. of suspension.....	2.9	9.1	12.4	10.3	162	7.10	6.85	6.92	6.95
5 cc. of suspension.....	3.3	8.9	10.6	12.1	83	7.17	6.87	7.13	7.25
Protozoan-filtrate.....						8.50			
Protozoan-suspension....						8.50			

Proteins may act either as an absorbing agent rendering iron more available or they may carry vitamins or vitamin-like substances. When 200 cc. of a Colpoda-Azotobacter culture was concentrated and ashed, even the addition of 1 gm. of the ash failed to stimulate; hence, the stimulating factor is organic.

Heat-killed cultures of *Azotobacter* or Seitz filtrate when added in varying concentrations were without visible effect. The stimulating substance produced by the protozoa was inactivated by autoclaving for  $2\frac{1}{2}$  hours at 15 pounds. Hence we may conclude that the stimulating substance elaborated by *Colpoda maupasii* is a complex organic substance heat-stable up to a certain degree but inactivated by prolonged heating and that it stimulates nitrogen fixation by causing the *Azotobacter* cells to reproduce more rapidly and over a longer period.

In order to make sure that the coexisting organisms did not fix nitrogen, the *Colpoda* were cultured for 14 days on heat-killed suspensions of *Escherichia coli* and *Aerobacter aerogenes*. No nitrogen gains occurred, and no mannite was used.

TABLE 6

*Nitrogen fixed and numbers of Azotobacter developed in liquid medium at 24°C. in the presence and absence of flagellates and flagellate-ciliate cultures*

	COLPONEMA SYMMETRICA	
	10 days	16 days
Nitrogen fixed, mgm.		
<i>Azotobacter</i> cultures.....	0.96	1.3
<i>Azotobacter</i> -flagellate cultures.....	1.57	1.9
<i>Azotobacter</i> -mixed protozoan cultures.....	5.1	6.3
Number of <i>Azotobacter</i> , millions/cc.		
<i>Azotobacter</i> cultures.....	40.7	37.1
<i>Azotobacter</i> -flagellate cultures.....	56.1	20.1
<i>Azotobacter</i> -mixed protozoan cultures.....	101.5	200.7

In two other experiments similar in outline to those previously given, the flagellate *Colponema symmetrica* and the ciliates *Colpoda maupasii* and *Oxytricha* sp.? were used as coexistent organisms. The results, given in table 6, show that the flagellate, like the amoeba, had little or no stimulating effect on nitrogen fixation whereas the large ciliate had a definite stimulating influence on both fixation and bacterial numbers.

#### *Experiments on agar media*

Agar plates were seeded with *Azotobacter*, and when a heavy growth appeared small numbers of *Colpoda maupasii* and *Hartmanella hyalina* were added. The plates were incubated at 20° to 30°C. in a moist chamber for 30 days. The results of three closely agreeing determinations are given in table 7. Both species of protozoa slightly increased nitrogen fixation. In many areas on the plates the surface bacterial growth disappeared completely, and in 1 week many of the protozoa were in a cystic condition.

*Experiments in sand culture*

Two triplicate experiments were conducted in which the organisms were grown in Petri plates containing sand. The coexistent organisms were *Colpoda maupasii*, *Hartmanella hyalina*, *Colponema symmetrica*. At the end of the incubation period the surfaces of the cultures containing the protozoa

TABLE 7

*Nitrogen fixed and numbers of organisms developed on agar media in the presence of Hartmanella and Colpoda*

	AT 20°		AT 30°	
	7 days	30 days	7 days	30 days
Nitrogen fixed, mgm./25 cc.				
Azotobacter cultures.....	2.0	2.5	2.9	5.2
Azotobacter-Colpoda cultures.....	3.0	3.6	4.6	5.8
Azotobacter-amoebae cultures.....	2.6	2.7	2.9	5.6
Azotobacter, millions,/cc.				
Azotobacter cultures.....	285.2		259.7	
Azotobacter-Colpoda cultures.....	282.0		95.3	
Azotobacter-amoebae cultures.....	204.3		323.0	
Amoebae, thousands/cc.....	76.1		255.3	
Colpoda, thousands/cc.....	179.7		205.4	

TABLE 8

*Nitrogen fixed, mannite consumed, and number of bacteria and the pH developed in sand cultures at 24°C.*

CULTURES	INCUBATED FOR 25 DAYS*		INCUBATED FOR 35 DAYS*		
	Bacteria	N fixed	N fixed	Mannite consumed	pH of medium
	millions/cc.	mgm.	mgm.	mgm.	
Azotobacter.....	97.85	4.78	2.7	332.2	7.68
Azotobacter-amoebae.....	13.87	4.19	3.5	369.2	7.77
Azotobacter-Colpoda.....	80.52	6.17	4.0	569.5	7.55
Azotobacter-flagellate.....	78.73	7.49	3.7	502.5	7.45

\* The 25- and 35-day periods represent two distinct experiments.

were orange-brown, whereas the control cultures showed only slight discoloration. Data obtained are given in table 8.

The protozoa caused a nitrogen increase of approximately 25 to 35 per cent, the greatest increase occurring in the presence of the ciliates and flagellates. Bacterial numbers were highest in the control and agree fairly well with the results obtained on agar media. The protozoan counts were 10,000 *Colponema*, 7,500 *Colpoda*, and 5,000 amoebae per cubic centimeter.

In a second sand experiment lasting 25 days the amoebae depressed nitro-

gen fixation, and the ciliates and flagellates stimulated nitrogen fixation to an even greater extent. Mannite consumption was substantially greater, with the exception of the amoebae cultures, in the presence of the protozoa than in the controls. The pH of the medium at the beginning was 7.75. The pH of the control cultures was reduced slightly, that of the amoebae-Azotobacter cultures not at all, and that of the Colpoda-Azotobacter cultures to a greater extent than the controls. In the flagellate-Azotobacter cultures the surface growth was purple-black, whereas in the controls only a slight yellow-orange color appeared. Protozoan numbers in all cases were 10,000 or more per gram.

TABLE 9

*Nitrogen fixed and the number of Azotobacter and protozoa developed in unmanured soil with and without mannite after 38 days at 24°C.*

	SOIL AND MANNITE		SOIL ALONE	
	1 w. h. c.*	$\frac{1}{2}$ w. h. c.	1 w. h. c.	$\frac{1}{2}$ w. h. c.
Nitrogen fixed, mgm./100 gm. of soil				
Azotobacter cultures.....	3.8	10.1	4.4	5.4
Azotobacter-amoebae cultures.....	9.1	12.1	3.4	1.2
Azotobacter-Colpoda cultures.....	7.9	10.1	5.5	6.7
Azotobacter, millions/gm.				
Azotobacter cultures.....	261	612	202	293
Azotobacter-amoebae cultures.....	255	484	218	186
Azotobacter-Colpoda cultures.....	319	468	325	176
Protozoa, thousands/gm.				
Amoebae.....	5.0	5.0	0.0	0.25
Colpoda.....	10.0	5.0	10.0	1.0

\* w. h. c. = water-holding capacity.

### *Experiments in soil*

Several experiments were conducted in sterilized soils of varying moisture and organic matter content. The organisms were cultured in the natural soil and in soil to which 1 per cent of mannite had been added. Cutler and Dixon (10) have found that  $\frac{1}{3}$  to  $\frac{1}{2}$  water-holding capacity is adequate soil moisture for trophic existence of the ciliates and that organic matter encourages protozoan activity.

In the first experiment unmanured soil from the Greenville experimental farm containing 1 per cent organic matter was used. Soil moisture was maintained at water-holding capacity and at  $\frac{1}{2}$  water-holding capacity. *Hartmanella hyalina* and *Colpoda maupasii* were used as coexistent organisms. They were incubated 38 days, after which nitrogen and numbers of microorganisms were determined (table 9). Because of the large quantity of organic matter in the soil which had been rendered available to the Azotobacter by the



7-hour sterilization at 117°C., nitrogen fixation was high in the soil without mannite. It was generally increased by Colpoda, though not to the same extent as in liquid media. The higher moisture content apparently favored

TABLE 10

*Nitrogen fixed and the number of Azotobacter and protozoa after an incubation period of 15 days at 24°C. in manured soil at two-thirds water-holding capacity*

	SOIL AND MANNITE		SOIL ALONE	
	Tumblers	Plates	Tumblers	Plates
Nitrogen fixed, mgm./100 gm. of soil				
Azotobacter cultures.....	8.2	12.1	2.0	2.4
Azotobacter-Colpoda cultures.....	15.4	7.3	6.1	2.4
Azotobacter-flagellate cultures.....	6.1	9.2	1.0	5.4
Azotobacter, millions/gm.				
Azotobacter cultures.....	745	745		190
Azotobacter-Colpoda cultures.....	845	2195	140	185
Azotobacter-flagellate cultures.....	700	1130	315	405
Colpoda, thousands/gm.....	10.0	10.0	10.0	10.0

TABLE 11

*Nitrogen fixed, number of Azotobacter, and the pH after 27 days at 24°C. in manured and unmanured soil at two-thirds water-holding capacity*

	MANURED SOIL				UNMANURED SOIL	
	Soil and mannite		Soil alone		Soil and mannite	Soil alone
	Tumblers	Plates	Tumblers	Plates	Tumblers	Tumblers
Nitrogen fixed, mgm.						
Azotobacter cultures.....	5.10	11.5	3.8	3.9	8.50	4.50
Azotobacter-Colpoda cultures.....	10.19	19.35	3.5	7.5	11.15	8.69
Azotobacter-amoebae cultures.....	10.14	18.5	3.4	5.1	10.00	4.34
Azotobacter-flagellate cultures.....	14.44	19.7	3.1	4.0	18.72	....
pH of:						
Uninoculated soils.....	7.45	7.20	7.75	....	7.55	7.75
Azotobacter cultures.....	7.65	7.40	7.70	7.45	7.70	7.90
Azotobacter-Colpoda cultures.....	7.77	7.40	7.50	7.55	7.70	7.85
Azotobacter-amoebae cultures.....	7.75	7.25	....	....	7.70	7.80
Azotobacter-flagellate cultures.....	7.70	7.50	7.80	7.65	7.80	....

Colpoda, but because of anaerobic conditions it was less favorable for nitrogen fixation. The amoebae, unlike the Colpoda, increased nitrogen fixation in both soil moistures in the presence of mannite but depressed fixation in the absence of mannite. The higher moisture content is unfavorable to the bac-

teria. The Colpoda-Azotobacter cultures contained greater numbers of bacteria than the controls at water-holding capacity both in the presence and in the absence of mannite, and at the lower water content the numbers were greater in the controls. This is in keeping with the findings of Greaves and Carter (15) as to the water requirements of Azotobacter. The amoebae, as in many of the other experiments, reduced bacterial numbers below that of the controls.

Another soil was used which contained 3 per cent of organic matter, as determined by the Schollenberger method. The moisture was adjusted to  $\frac{2}{3}$  water-holding capacity, as a compromise between unit capacity, more favorable to the protozoa, and  $\frac{1}{2}$  the capacity, more favorable to the Azotobacter. Colpoda and Colponema were the coexistent organisms. Large Petri plates and tumblers were used. The results are reported in table 10. Stimulation of nitrogen fixation by Colpoda was positive both in the presence and in the absence of mannite in the tumblers, whereas only the flagellates caused an increase in the plate cultures. Although mannite influenced fixation, humus had little effect. Azotobacter numbers in the soils containing mannite in the Colpoda-Azotobacter cultures were higher than in the controls.

Both of the previous experiments were repeated with soil cultures of known pH. *Colpoda maupasii*, *Hartmanella hyalina*, and *Colponema symmetrica* were used as the coexistent organisms. Results are given in table 11. Nitrogen fixation in the Azotobacter-protozoa cultures containing mannite was decidedly stimulated in all cases, irrespective of the kind of soil. The Azotobacter organisms without an available energy supply were not able to maintain themselves in the face of the phagocytic activity of the protozoa. The numbers of bacteria were substantially greater in both the tumbler and the plate cultures containing mannite in the presence of the protozoa than they were in the controls. The reactions of the cultures were not visibly different, probably because of the highly buffered soils used. Fedorowa-Winogradowa (13), however, has noticed that some soil cultures containing protozoa became more alkaline.

#### DISCUSSION

Previous theories concerning the mechanism by which protozoa stimulate nitrogen fixation, namely, the phagocytic theory of Cutler et al. and the disjunctive symbiotic theory of Hirai and Hino, are not adequate to explain the results obtained in these experiments. The larger numbers of Azotobacter cells found in the presence of the protozoa and the inability of the protozoa to maintain an optimum reaction for the Azotobacter during the most intense period of fixation point to some other factor or perhaps factors responsible for the stimulation. The fact that killed suspensions of protozoa will also stimulate fixation and cell division in Azotobacter points to a nutritional factor. Moreover the fact that relatively small concentrations of the material are adequate for stimulation indicates that the substance may be enzymatic or

vitaminlike in nature. Certainly it is organic and complex, for the charred material or a Seitz filtrate is without effect.

Cutler and Bal (9) found that amoebae, as a rule, exert less stimulation on *Azotobacter* than do the ciliates, and may even fail to exert any stimulation. Fedorowa-Winogradowa (13) found 37 per cent increase in nitrogen fixation due to amoebae. Previous to our investigation, no study of flagellates in connection with nitrogen fixation is known. Kubiena (24), Sandon (37, 38) and others (7, 26, 27) have demonstrated that ciliates are active in field soils that are sufficiently moist to support most crops. Ciliates are not numerous in the soil, but the fact that they need be present in only small numbers to cause stimulation may be significant.

The results as to nitrogen fixation corroborate those reported by Cutler (9) and co-workers (29), Hirai and Hino (21), Fedorowa-Winogradowa (13), Lander (25), and Hill (20).

Especially significant is the fact that the protozoa stimulate nitrogen fixation markedly in the soil itself, provided the bacteria have a sufficient supply of energy material. Although sterilized soil, which differs from natural soils with their myriads of competing organisms, was used as a culture medium, nevertheless the results indicate that some protozoa, under proper conditions, may increase nitrogen fixation in natural soils.

#### SUMMARY

*Azotobacter chroococcum* was cultured in sand, soil, agar, and liquid media together with various species of bacteria-free protozoa to determine the effect of these organisms on nitrogen fixation.

The protozoa generally stimulated nitrogen fixation. The greatest stimulation was obtained in liquid media using ciliates as the coexistent organisms. Very little stimulation of nitrogen fixation was obtained on agar media; in many cases nitrogen fixation was depressed. Stimulation was slight but definite in sand culture in the presence of ciliates, flagellates, and amoebae. Nitrogen fixation was stimulated by four coexistent species of protozoa in soil cultures containing mannite, but in many instances it was depressed in soil without mannite.

In both liquid and soil media, *Azotobacter* were more numerous in the presence of the protozoa than in their absence. The cells in the *Azotobacter*-protozoa cultures were relatively small and stained deeply, and the pellicles were extremely thick and leathery.

The ciliates (Colpoda and Oxytricha), and the flagellates and amoebae to a less degree, produce a substance which favors *Azotobacter*. Suspensions of Colpoda, heat-killed at 65°C. for one-half hour, when introduced into *Azotobacter* cultures in small amounts, stimulated nitrogen fixation in a manner similar to that of the living protozoan cells. This substance, which is not destroyed by low heat but is inactivated by prolonged heating in the autoclave, need be present in only minute quantities, since the presence of a few

hundred cells per cubic centimeter is sufficient to bring about stimulation equivalent to that of 20 to 40 thousand protozoan cells per cubic centimeter. Seitz filtrates of the substance have no stimulating effect, indicating that the substance may be an organic colloid held back by the filter.

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# THE COLLOIDAL CONSTITUENTS OF AMERICAN ALKALI SOILS

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As is well known, the degree of Na saturation and the concentration and specific kind of soluble salts present in alkali soils are extremely variable. The pH of such soils is commonly above 7.0, sometimes markedly so, and the clay is often found to be highly dispersed. Although alkali soils have been extensively investigated, but little is known about their colloidal constituents. The conditions which prevail in these soils might conceivably bring about some alteration in the clay that was present before the soluble salts accumulated, or else the conditions might exert a modifying influence on the formation of clay. On the other hand, the effects of the alkali condition may be limited to the replacement of ions from the surface of soil particles, alterations in pH, and dispersion of the clay, the last named being the usual result of replacement of divalent cations by Na.

In 1937, Antipov-Karataev and Sedletzky<sup>1</sup> (2) announced the synthesis of a new clay mineral which they named "gedroizite," in honor of the late K. K. Gedroiz. Recently Sedletzky reported that gedroizite is the chief component of the 0.2-2.0  $\mu$  colloid of the horizon of certain Russian alkali soils which show the so-called solonetz morphology (19). On the other hand, colloidal particles of this horizon less than 0.2  $\mu$  in diameter were found to be montmorillonitic. Since he found that the very thin eluvial horizon (0.0-0.5 cm.) above the solonetz horizon, and also the materials below this horizon, contain not gedroizite, but montmorillonite, Sedletzky concluded that the peculiar chemical conditions which prevail within the solonetz horizon (high pH and Na saturation) are responsible for the formation of gedroizite. In contrast to the solonetz horizon of alkali soils, Sedletzky (18) found that the clays of several Russian saline soils are most commonly montmorillonitic in type, although he reported other clay minerals in certain samples.

## SOILS STUDIED

The soils used in this investigation were drawn from several different localities in western United States. Since the profile of American alkali soils is, with minor exceptions, only feebly developed, the samples were taken to a depth of 12 inches, except as otherwise stated. The majority of the soils

<sup>1</sup> Also spelled Sedleckij.

used contain substantial amounts of soluble Na salts, seven of the following being of the black-alkali type, and three of the white-alkali type:

1 [19012]<sup>2</sup>. Fresno fine sandy loam, 0-12 inches, taken 9 miles southwest of Fresno, California. The alkali condition of this soil developed between 1900 and 1920, when, because of a high water table which existed during that period, soluble salts rose by capillarity from the deep subsoil. The soluble salts consist of NaCl, Na<sub>2</sub>SO<sub>4</sub>, and Na<sub>2</sub>CO<sub>3</sub>. This soil is alluvial, derived largely from granite.

2 [19013]. Fresno fine sandy loam, 0-12 inches, similar to sample 1, taken from a nearby gypsum-treated plot of the alkali-reclamation experimental area of the University of California. The reclamation of this plot has been completely effective, all apparent evidences of the previous alkali conditions having been obliterated by the gypsum treatment.

3 [7925]. Imperial clay, 0-12 inches, taken from a virgin soil area in the Imperial Valley, 3 miles west of Imperial, California.

4 [7926]. Holtville clay, 0-12 inches, taken from a virgin soil area in the Imperial Valley, 1 mile north of Seeley, California.

5 [18218]. Holtville clay loam, 0-12 inches, taken from a previously cultivated area in the Imperial Valley, 3 miles east of El Centro, California.

Samples 3, 4, and 5 contain high concentrations of NaCl, Na<sub>2</sub>SO<sub>4</sub>, and CaSO<sub>4</sub>, and also considerable MgCl<sub>2</sub> and MgSO<sub>4</sub>. They are typical white-alkali soils, such as occur in many places in the Imperial Valley of California. These soils are alluvial, derived chiefly from shales and sandstones.

6 [8461]. Rosamond clay, 0- $\frac{1}{2}$  inch, taken from a dry lake bed in the Mojave Desert about 15 miles northeast of Lancaster, California. This sample represents very recent alluvium, such as is washed into certain shallow basins in the Mojave Desert by the occasional heavy rains which fall there. It contains only moderately high concentrations of NaCl and Na<sub>2</sub>CO<sub>3</sub>. The clay was probably derived chiefly from the surrounding black-alkali-soil areas across which the streams flow in passing to the lake bed. The surrounding soils are alluvial, derived chiefly from granite, but partly from basic rocks, shales, and sandstones.

7 [5695]. Fallon silt loam, 0-12 inches, taken near Fallon, Nevada. This soil contains relatively low concentrations of soluble Na salts and is extremely dispersed. It is an alluvial black-alkali soil, derived chiefly from granite.

8 [7081]. Unnamed silt loam, 0-12 inches, taken near Ontario, Oregon, from an area of virgin soil adjacent to the Vale alkali-reclamation experiment of the Oregon Agricultural College. This sample represents an extreme type of virgin black-alkali soil. It contains an unusual combination of soluble Na salts (10) and is alluvial from eruptive rocks.

9 [15607]. Sunrise silty clay, 0-12 inches, taken from Sec. 20, T 1 N, R 4 E, near Salt River, about 1 mile from Tempe, Arizona. This soil contains considerable NaCl, Na<sub>2</sub>SO<sub>4</sub>, and Na<sub>2</sub>CO<sub>3</sub>. It is an alluvial black-alkali soil derived chiefly from granite.

10 [15577]. Unnamed clay loam, 0-12 inches, taken from Sec. 32, T 10 N, R 3 W, of the Corinne Drainage District about 11 miles west of Brigham City, Utah. This sample contains considerable NaCl and Na<sub>2</sub>CO<sub>3</sub>. The area sampled was formerly submerged by Lake Bonneville, but the surface-soil materials were probably laid down since the lake receded from this area. The alluvium was probably derived from a variety of rock formations, among which are the calcareous sandstones of the Salt Lake Formation and the limestone, dolomitic limestone, and shales of the Wasatch Mountains.

11-15. Solonetz soils, description and discussion of which are deferred to the section "Solonetz colloids."

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<sup>2</sup> With the exception of sample 15, the soils used in this work were selected from the large series of soil samples of the Citrus Experiment Station. The bracketed numbers given are the Citrus Experiment Station laboratory numbers of these samples.

The colloids were separated from the coarser soil materials of the samples by sedimentation so adjusted as to secure particles of  $1\ \mu$  maximum diameter. The clay suspensions were filtered through Chamberland-Pasteur tubes, after which the colloids were dried at room temperature, then passed through a 60-mesh sieve.<sup>3</sup> In the air-dry form these colloids were grayish white and low in organic matter. Samples 3, 4, and 5 were artificially saturated with Ca by leaching with a normal solution of Ca acetate. All other samples were analyzed without chemical treatment.

## CHEMICAL ANALYSIS

Table 1 gives the results of fusion analysis of these colloids. The data show that these alkali-soil colloids differ from the other soil colloids previously

TABLE 1  
*Chemical analysis of alkali-soil colloids*

LABORATORY NUMBER	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	TiO <sub>2</sub>	MnO	P <sub>2</sub> O <sub>5</sub>	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	SO <sub>2</sub>	CO <sub>2</sub>	H <sub>2</sub> O	TOTAL
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	50.55	17.59	8.82	0.86	0.10	0.10	2.63	4.34	3.84	2.04	0.07	0.63	9.13	100.70
2	53.56	16.98	8.80	0.85	0.09	0.10	2.15	3.46	3.76	2.01	0.07	0.08	8.60	100.51
3	51.20	19.30	6.51	0.76	0.05	0.09	1.70	3.71	3.06	0.39	0.02	0	14.00	100.79
4	50.22	18.47	6.57	0.66	0.07	0.26	2.02	3.90	2.48	0.32	0.06	0	14.64	99.67
5	50.12	18.36	6.65	0.66	0.08	0.30	1.63	3.73	2.53	0.28	0.02	0	14.91	99.27
6	41.72	15.23	8.20	0.63	0.11	0.48	5.22	6.73	2.62	2.91	0.03	3.65	10.88	98.41
7	49.36	15.57	7.02	0.82	0.36	0.32	6.02	1.71	2.81	1.94	0.08	3.42	10.58	100.01
8	57.50	11.72	6.76	0.82	0.34	0.30	3.72	0.71	2.64	2.48	0.06	2.27	11.79	101.11
9	44.57	14.92	6.38	0.65	0.31	0.38	6.89	1.74	3.07	1.49	0.07	7.42	9.67	97.56
10	44.15	13.30	4.72	0.56	0.20	0.30	10.98	4.26	3.65	0.92	0.06	8.13	9.40	100.63

investigated by this laboratory (11, 12) in that their content of SiO<sub>2</sub> and CaO is relatively high. The results reported in table 2, together with those of Brown and Byers (3), suggest that relatively high SiO<sub>2</sub> is characteristic of the colloids of dry-land soils generally. The exceptionally high CaO content of samples 6 to 10, inclusive, was undoubtedly due largely to CaCO<sub>3</sub>. The MgO content was variable, being unusually low in sample 8 from Oregon, and very high in sample 6 from the Mojave Desert. The K<sub>2</sub>O content was of the same order of magnitude as that in colloids from nonalkali, dry-land soils (3).

The black-alkali-soil colloids were found to contain more or less carbonate (CO<sub>2</sub>). As will be shown in the x-ray section of this paper, calcite is a constituent of these colloids. Samples 6, 9, and 10, however, must also contain

<sup>3</sup> Thanks are extended to S. M. Brown for separating all the colloids reported herein except no. 15.



small amounts of some other carbonate, since the CaO equivalent of the  $\text{CO}_2$  found exceeds the total CaO of these samples.

Artificial Ca saturation of colloids, 3, 4, and 5 may have reduced their content of Na, but this is not the main reason for their low content of  $\text{Na}_2\text{O}$ . It is more probable that the type of alluvium deposited in the Imperial Valley was low in insoluble Na originally. Most of the other samples were from soils which have been derived from very different types of alluvium.

### *Silica-sesquioxide ratios*

As shown in table 2, these colloids are characterized by high  $\text{SiO}_2/\text{Al}_2\text{O}_3$  and  $\text{SiO}_2/\text{R}_2\text{O}_3$  ratios, the former being well above 4 in all cases and extraordinarily high—8.34—in sample 8. The soil from which this later colloid

TABLE 2  
*Molecular ratios and exchangeable bases of alkali-soil colloids*

LABORATORY NUMBER	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{R}_2\text{O}_3}$	EXCHANGE- ABLE Na	BASE-EX- CHANGE CAPAC- ITY	Na SATURA- TION	pH OF ORIGINAL SOIL
			<i>m.e.</i>	<i>m.e.</i>	<i>per cent</i>	
1	4.88	3.70	7.6	25.7	29.5	10.25
2	5.37	4.03	1.2	26.1	4.6	8.70
3	4.51	3.71	0	42.7	0	8.05
4	4.55	3.72	0	46.9	0	8.10
5	4.56	3.72	0	49.9	0	8.50
6	4.51	3.35	46.8*	33.9	100.0	9.65
7	5.39	4.18	2.3	37.5	6.1	9.40
8	8.34	6.10	50.4	58.4	86.3	10.85
9	5.07	3.99	13.2	34.3	38.5	9.15
10	5.64	4.61	19.6	26.1	75.1	9.50

\* This sample is peculiar in that the exchangeable Na apparently exceeds the base-exchange capacity. This was probably due to solubility effects.

was separated was previously shown to contain unusual amounts of soluble silicate (10). This colloid probably contains considerable colloidal  $\text{SiO}_2$ . Brown and Byers (3) also found high  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios for the colloids of certain dryland soils; these, however, were probably not alkali soils.

### *Base-exchange capacity and replaceable Na*

The base-exchange capacity of these colloids (determined by the  $\text{NH}_4$ -acetate method) varied from 25.7 to 58.4 m.e. It is noteworthy that, whereas colloids 3, 4, and 5, which as shown in the x-ray section of this paper are montmorillonitic, are intermediate in base-exchange capacity, sample 8, from Vale, Oregon, the highest found in base-exchange capacity, gave no x-ray evidence of montmorillonite. Recently it was shown (12) that the kaolinitic Vina colloid is also high in base-exchange capacity. Again it is shown that the

relation between base-exchange capacity and  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio is not always close. Several different Si- and Al-containing constituents of variable composition and variable base-exchange power were probably present in different proportions in these colloids. It is possible that an unusual part of the total base-exchange capacity of colloid 8 from Oregon was due to amorphous material.

As shown in table 2, the percentage Na saturation varied widely in these colloids. Samples 6, 8, and 10 are highly saturated with Na, whereas sample 7 contains but little replaceable Na. The low content of exchangeable Na in sample 2 was due to the artificial application of gypsum previously referred to;

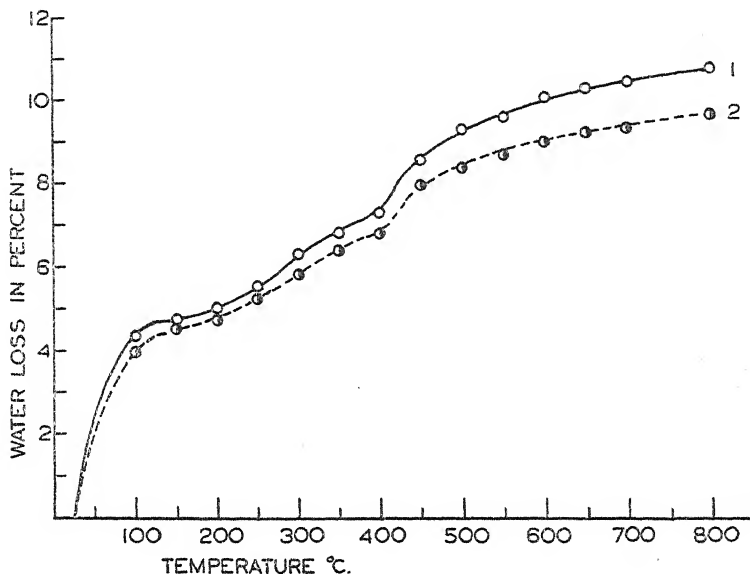


FIG. 1. DEHYDRATION CURVES OF RECENTLY FORMED BLACK-ALKALI-SOIL COLLOIDS FROM FRESNO, CALIFORNIA

the high concentration of soluble Ca and Mg present in soils 3, 4, and 5 probably prevented the natural absorption of very much Na by the colloids of these soils, and that which was absorbed was removed artificially by Ca saturation in the laboratory.

#### DEHYDRATION INVESTIGATIONS

The colloid samples were first brought to equilibrium with the atmosphere above a 50 per cent solution of  $\text{H}_2\text{SO}_4$  at  $25^\circ\text{C}$ ., then heated to constant weight at  $50^\circ$  intervals of temperatures up to  $800^\circ\text{C}$ . The losses in weight were plotted against temperature. Figure 1 gives the results for samples 1 and 2, which are from the black-alkali soil of Fresno, California. Figure 2 refers to the white-alkali-soil colloids 3, 4, and 5. These latter curves closely

approximate the curve for the Yolo-soil colloid (12), which is composed largely of montmorillonitic clay; as will be shown later, these white-alkali-soil colloids also contain montmorillonite. As the curves show, the low-temperature water of the white-alkali-soil colloids (fig. 2) is considerably greater than that of the black-alkali-soil colloids (figs. 1 and 3), which contain very little montmorillonite. As Kelley, Jenny, and Brown (9) showed, the loss of a high percentage

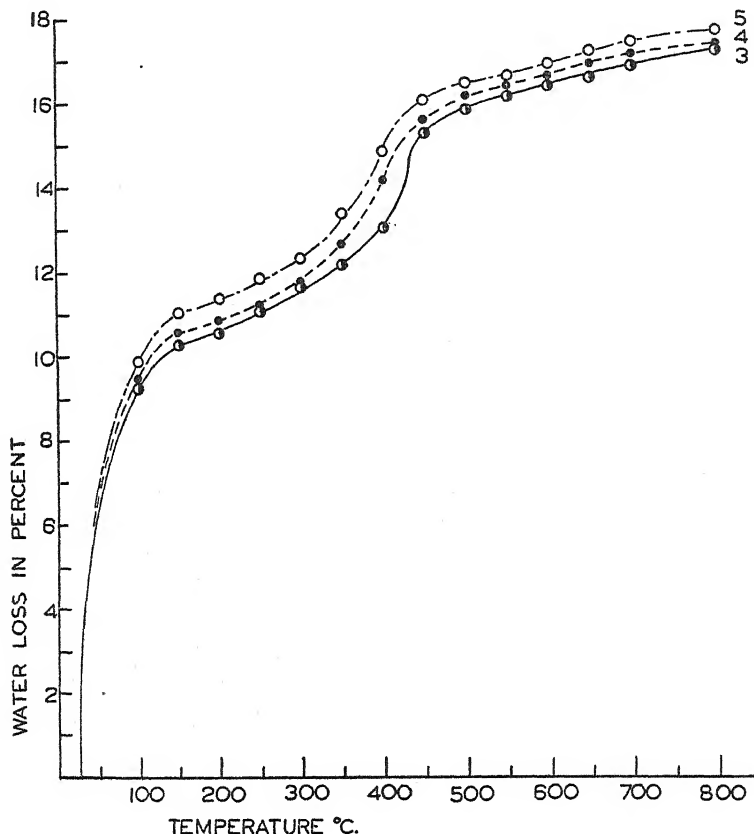


FIG. 2. DEHYDRATION CURVES OF WHITE-ALKALI-SOIL COLLOIDS FROM IMPERIAL VALLEY, CALIFORNIA

of its total water content at 150°C. appears to be characteristic of montmorillonite.

The dehydration curves of black-alkali-soil colloids 6 to 10 (fig. 3) are very different in shape. They show pronounced breaks beginning just above 400°C. This would ordinarily indicate kaolinitic material, but the x-ray evidence fails to support this indication. Much of the loss in weight beginning at approximately 400° was probably due to the evolution of  $\text{CO}_2$  from  $\text{CaCO}_3$ . In fact, these samples sustained losses in weight between 400° and 500° approximately

equivalent to carbonate content, although decarbonation of  $\text{CaCO}_3$  normally requires a higher temperature. As will be shown later, the x-ray measurements indicate that the calcite of these samples was largely decomposed at  $500^\circ\text{C}$ . It is possible that crystalline calcite, when in particles of colloidal

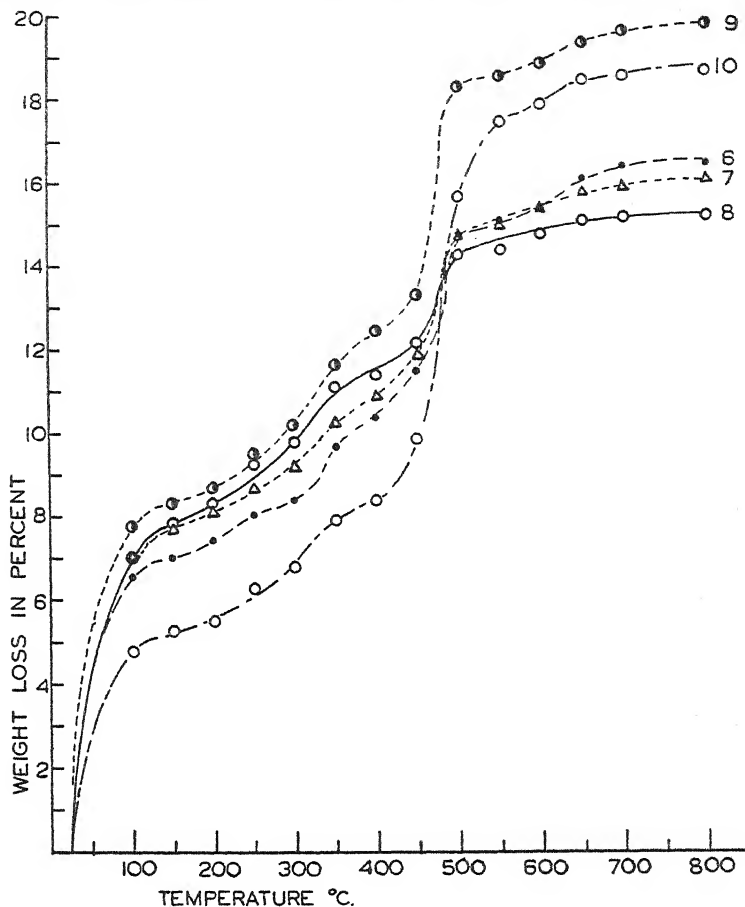


FIG. 3. DEHYDRATION CURVES OF NATURAL BLACK-ALKALI-SOIL COLLOIDS FROM VARIOUS STATES

6, Mojave Desert; 7, Fallon, Nevada; 8, Vale, Oregon; 9, Salt River Valley, Arizona; 10, Corinne Drainage District, Utah

size, gives up  $\text{CO}_2$  at a somewhat lower temperature than does ordinary calcite. It is also possible that colloidal  $\text{CaCO}_3$  reacted at elevated temperatures with some other constituent of the samples with the consequent loss of  $\text{CO}_2$ .

#### X-RAY INVESTIGATIONS

The colloids were x-rayed in two forms, namely, air-dried and after being heated to constant weight at  $500^\circ\text{C}$ . By this means it is possible more defi-

nately to interpret the x-ray films, as was discussed in a previous paper (12).

X-ray diffraction patterns were obtained by packing the colloids in thin-walled soft-glass tubes of about 0.4 mm. diameter and exposing them to  $K\alpha$  molybdenum radiation filtered through a zirconium oxide screen. It was at once apparent from the x-ray films that the mineral composition of these alkali-soil colloids is different in certain respects from that of the nonalkali-soil

TABLE 3  
*Theoretical interplanar spacings for calcite*

SPACING d	INTENSITY*	MILLER INDEXES
Å.		
3.85	v.w.	(110)
3.03	v.s.	(112)
2.49	w-m.	(110)
2.28	m.	(120)
2.09	m.	(200)
1.92	m.s.	(220)
1.87	m.s.	(123)
1.61	m.	(133)
1.52	m.	(130)
1.47	w.	(230)
1.45	m.	(344)
1.42	w.	(444)
1.34	v.w.	(124)
1.292	w.	(134)
1.25	v.w.	(335)
1.23	v.w.	(345)
1.175	w.	(235)
1.153	w.	(144)
1.045	w.	(400)
1.013	v.w.	(334)
.960	v.w.	(440)
.942	v.w.	....

\* Symbols for the relative intensities of the x-ray reflections: v.s. = very strong; s. = strong; m.s. = medium strong; m. = medium; w-m. = weak to medium; w. = weak; v.w. = very weak; v.v.w. = very, very weak; tr. = trace; ? = doubtful.

colloids which were examined previously in this laboratory. Though the clay minerals found in normal soils; namely, kaolinitic, montmorillonitic, and micalike clays,<sup>4</sup> were also found in these soils, a considerable number of lines which have not been reported for normal-soil colloids were noted on the films.

As pointed out already, the chemical analyses (table 1) showed the presence of considerable Ca and  $CO_3$  in some of the samples, and this suggested that crystalline calcite might be present. A comparison of the x-ray films with

<sup>4</sup> Referred to in a previous paper (12) as illite or x-mineral.

those produced both by ground calcite and by precipitated  $\text{CaCO}_3$  showed that a number of lines were common to all of them. Accordingly, a set of reference spacings was calculated for calcite using the established hexagonal unit cell with  $a = 6.36\text{\AA}$ . and  $\alpha = 46^\circ 6'$  (21, p. 273). These are listed in table 3. Those used for the identification of the clay minerals and quartz were published in a previous paper (12, table 20).

Tables 4, 5, 6a, and 6b show the values of all the interplanar spacings that could be measured with certainty, together with visual estimates of the relative intensities of the x-ray lines. The first column of each table presents letters indicating the mineral that might have produced the opposite spacing. With such heterogeneous material, the significance of any single spacing must be accepted with caution. It was found convenient to segregate the data into four tables corresponding to the natural groups into which these colloids fall.

#### *White-alkali-soil colloids*

The x-ray patterns of the white-alkali-soil colloids (table 4) do not differ greatly from those of previously studied colloids from certain nonalkali soils. There is evidence in these samples of montmorillonitic, kaolinitic, and micalike clay minerals, as well as quartz. Also a number of unidentified lines were noted. Montmorillonite was indicated by the  $15\text{--}16\text{\AA}$ . spacing in all three of the unheated samples, and by the  $10\text{\AA}$ . spacing after heating to  $500^\circ\text{C}$ . The intensity of the line corresponding to this spacing in the heated material may have been partly contributed to by the micalike clay, the presence of which was clearly indicated by the medium to strong  $2.57\text{--}2.60\text{\AA}$ . spacing. This idea is not necessarily contradicted by the absence of a  $10\text{\AA}$ . line in the patterns of the unheated materials, since the micalike clay usually gives a weak  $10\text{\AA}$ . line. Two of the samples contain some kaolinitic material, as shown by the  $7.0\text{\AA}$ . line which disappeared on heating. Quartz is strongly indicated by six definite quartz lines in all three samples.

In addition to the spacings which appear to be definitely related to the aforementioned minerals, the patterns of the white-alkali-soil colloids contain a number of lines that we have thus far been unable to assign to any known mineral. Especially conspicuous among these is a group of narrow spacings (below  $1.23\text{\AA}$ .), which are not usually observed in normal soil colloids. Some of these spacings are close to those of calcite, but calcite is definitely absent from these samples, as shown by chemical analysis and also by the absence of a very strong  $3.03\text{\AA}$ . line. In a further attempt to discover what crystal-line constituent was responsible for the unidentified lines, attention has been given to the possibility that some mineral related to the synthetic gedroizite, referred to by Antipov-Karataev and Sedletzky (2) and by Sedletzky (19), may have been present. It appears from Sedletzky's discussion that gedroizite occurs in those horizons of alkali soils which show the solonetz morphology and which are high in pH and exchangeable Na and comparatively low in soluble salts. It is more likely, therefore, to be found in black-alkali

soils than in white-alkali soils. The x-ray pattern of gedroizite, as given by Sedletzky, however, shows a number of narrow spacings similar to

TABLE 4  
*Interplanar spacings for white-alkali-soil colloids*

MINERALS*	3		4		5	
	Air-dried	Heated to 500°	Air-dried	Heated to 500°	Air-dried	Heated to 500°
	Å.	Å.	Å.	Å.	Å.	Å.
M	16.5 w.		15.2 w.		15.5 s.	
MI		10.0 v.w.		10.0 w.		10.0 v.w.
K	7.0 v.w.		7.2 w.			
M(U)			5.4 w.	5.1 v.w.	5.4 m.	
U					4.6 s.	4.6 s.
MI	4.5 s.	4.5 s.	4.5 s.	4.5 s.	4.5 s.	4.5 s.
Q		4.2 v.w.				
I	3.85 w.	3.70 v.v.w.		3.70 w.		3.7 v.w.
U	3.37 s.	3.38 v.w.				
Q	3.32 s.	3.35 s.	3.35 v.s.	3.35 v.s.	3.35 v.s.	3.35 v.s.
I	2.87 w.					
IMK	2.57 s.	2.57 m.	2.57 m.	2.57 s.	2.58 v.s.	2.57 v.s.
K	2.49 w.					
Q	2.45 w.	2.45 m.	2.47 v.w.	2.45 w.	2.47 w.	2.45 w.
Q	2.28 w.	2.27 m.s.	2.27 v.w.		2.28 v.w.	2.27 v.w.
IM				2.25 w.		
I	2.13 w.	2.11 w.	2.15 v.w.		2.15 v.w.	2.15 v.w.
I	1.98 m.	1.97 w.	2.01 v.w.	2.02 w.	1.99 v.w.	1.99 v.w.
Q	1.81 m.	1.81 s.	1.82 w.	1.82 w.	1.81 m.	1.81 m.
M	1.71 w.	1.71 m.	1.70 w.	1.70 w.	1.71 w.	1.73 w-m.
Q	1.67 w.	1.67 m.		1.66 w.	1.66 w.	1.65 w.
Q	1.54 w.	1.54 m.	1.55 w.	1.55 w.		1.57 w-m.
IM	1.50 v.s.	1.50 v.s.	1.50 s.	1.51 m.s.	1.50 s.	1.50 w.
KU		1.45 v.w.	1.46 w.	1.46 w.	1.46 v.w.	1.46 v.w.
Q	1.38 s.	1.375 v.s.	1.38 w.	1.38 m.s.	1.37 m.	1.38 m.
M	1.31 m.s.	1.31 s.	1.30 w.	1.31 w.		
I	1.29 w.	1.29 w.			1.29 m.	1.29 m.
M	1.25 w.	1.26 w.	1.26 w.	1.26 m.	1.25 w-m.	1.26 m.
U	1.20 v.w.	1.20 w.				
U	1.18 v.w.	1.18 w.				1.185 v.w.
U		1.08 v.w.				1.145 v.w.
U		1.06 v.w.				
U		1.01 v.w.				
U		0.99 v.w.				

\* Symbols for minerals: C = calcite; I = micalike clay mineral; K = Kaolinite; M = montmorillonite; Q = quartz; U = unidentified mineral. These letters signify that the particular line in the pattern might be due to the indicated mineral, not that the presence of the mineral is necessarily established by the line.

See footnote table 3 for explanation of intensity symbols.

those observed in these white-alkali soil colloids. This fact made it desirable to compare the x-ray data with those given for gedroizite.

In the text Sedletzky stated that the most characteristic spacings for gedroi-

zite are 3.69Å., 3.21Å., 1.60Å., and 1.02Å., but it is to be noted that only two of these lines are given the same numerical values in his tabulated list (19). Only one line in the reference list, namely, the 3.21Å., is claimed to be strong. Ten lines are designated as medium, and they include the 1.02Å. line and two that are fairly close to the 3.69Å. and 1.60Å. lines. Since crystal-structure analysis has not been made on Sedletzky's synthetic product, there is no certainty that the series of lines reported by him were produced by a single homogeneous mineral. The optical data and other evidences given by him suggest rather strongly that his preparation was decidedly heterogeneous. Until this material can be shown to be a homogeneous crystalline individual and some plausible unit cell consistent with the available data can be assigned to it, a reliable set of reference spacings cannot be said to be established.

In spite of these uncertainties, however, it is instructive to compare the unidentified spacings of the white-alkali-soil colloids with those reported for gedroizite. Among the narrower spacings, there is reasonably close agreement with Sedletzky's list. Since within the range under consideration, Sedletzky's list covers many spacings separated by narrow steps which little more than span the experimental error of measurement, the agreement found might be fortuitous and without interpretative significance. The wider unidentified spacings given by these colloids have no counterpart in Sedletzky's list. Especially significant is the complete absence of any spacing closely approaching the 3.21Å. line which Sedletzky reported to be outstandingly strong in the gedroizite pattern. There appears to be no good evidence, therefore, that gedroizite is a constituent of these white-alkali-soil colloids.

#### *Black-alkali-soil colloids*

The x-ray patterns for the black-alkali-soil colloids, as shown in tables 5, 6a, and 6b, differ distinctly from those of the white-alkali-soil colloids, and they also differ from many of the previously reported normal-soil colloids. One of the striking features of these colloids is found in the virtual absence of clay minerals of the montmorillonitic and kaolinitic types and the consistent presence of the micalike clay. Since it has been shown that Na-saturated colloids sometimes tend to give diffuse x-ray lines, samples were Ca-saturated and then x-rayed again, but there was no distinguishable difference between these films and those obtained originally.

In agreement with the chemical analyses, all of these black-alkali-soil colloids gave x-ray evidence for calcite, which is not a constituent of the normal-soil colloids previously reported from this laboratory (11, 12). These samples also showed the presence of quartz, and in addition there is some reason to believe that they contain amorphous  $\text{SiO}_2$ . A few unidentified lines occur in the patterns. The conclusion is, therefore, that these black-alkali-soil colloids consist chiefly of micalike clay, quartz, and calcite, together with smaller amounts of unidentified minerals and amorphous  $\text{SiO}_2$ . Sample 1 apparently contains minor amounts of montmorillonite also, since the unheated sample



gave a weak  $15\text{\AA}$ . line and a  $10\text{\AA}$ . spacing which was strengthened by heating to  $500^{\circ}\text{C}$ .

It is important to point out in this connection that the determination of

TABLE 5  
*Interplanar spacings for black-alkali-soil colloids*

MIN- ERALS*	1		2	
	Air-dried	Heated to $500^{\circ}$	Air-dried	Heated to $500^{\circ}$
	$\text{\AA}$ .	$\text{\AA}$ .	$\text{\AA}$ .	$\text{\AA}$ .
M	15.0 w.			
IM	10.2 w.	10.0 m.s.	10.0 w.	10.0 w.
MI	4.5 w-m.	4.5 m.	4.5 m.	4.5 v.v.w.
Q	4.25 w-m.	4.22 m.	4.2 m.	
I	4.08 w-m.	4.05 m.		4.1 v.w.
U			3.99 m.	
I	3.75 w-m.	3.72 w-m.	3.70 m.	
U			3.60 w.	
Q	3.35 v.s.	3.35 v.s.	3.32 v.s.	3.35 v.s.
I	3.20 v.s.	3.20 v.s.	3.20 s.	3.20 s.
C	3.00 m.	3.00 m.	3.00 w.	3.01 w.
I	2.98 w.	2.98 w.		
U	2.94 w.	2.94 w.		
U	2.62 m.	2.61 m.	2.60 m.s.	2.63 m.
I	2.57 w.	2.51 w.		
I	2.43 m.	2.42 m.	2.43 m.	2.43 m.
U			2.32 w.	2.30 w.
QC	2.28 w.	2.27 m.		
I	2.17 w.	2.17 w.	2.18 v.w.	2.20 w.
IC	2.10 v.w.			
I	1.98 w.	2.01 w.	2.01 w.	2.00 w.
C	1.92 v.w.		1.92 v.w.	
Q	1.83 m.	1.83 m.	1.82 v.w.	1.84 w.
U	1.75 w.	1.74 w.	1.74 w.	1.74 v.w.
Q	1.68 v.w.	1.67 m.	1.67 m.	1.68 w-m.
I	1.66 v.w.			
Q	1.54 m.s.	1.53 s.	1.54 m.s.	1.54 m.s.
I	1.49 v.w.		1.50 m.	
C	1.46 w.	1.46 w.	1.46 w-m.	1.46 w.
C	1.43 v.w.			
Q	1.38 m.	1.38 m.s.	1.38 m.	1.38-w-m.
U	1.35 v.w.	1.36 v.w.		
I		1.32 w.	1.32 v.w.	
I		1.25 w.		1.26 w.

\* See footnote table 4.

minor amounts of montmorillonitic clay in heterogeneous materials, like soil colloids, is difficult. Experiments with known mixtures of montmorillonite and other kinds of colloids have shown that as the proportion of montmoril-

lonite decreases the strength of the x-ray lines progressively diminishes. When the mixture contains not more than approximately 10 per cent montmorillonite, the x-ray lines sometimes become too weak to be measured with

TABLE 6a  
*Interplanar spacings for black-alkali-soil colloids*

MIN- ERALS*	6		7		8	
	Air-dried	Heated to 500°	Air-dried	Heated to 500°	Air-dried	Heated to 500°
	Å.	Å.	Å.	Å.	Å.	Å.
I	10.0 w.	10.2 v.w.	10.0 v.w.	10.0 w.		10.8 w.
U	5.15 m.s.					5.1 v.w.
I	4.5 s.	4.5 s.	4.55 w.	4.55 w.	4.55 s.	4.55 m.s.
I			4.10 w.	4.06 m.		4.15 m.s.
U			3.98 w.	3.74 w-m.	4.00 m.	
CI		3.8 w.	3.72 w.		3.70 w.	3.85 v.w.
IQ	3.40 m.s.	3.35 v.s.	3.40 w.	3.30 s.	3.40 m.	3.40 v.s.
I			3.25 m.s.		3.29 m.	3.30 s.
I		3.20 w-m.		3.15 v.s.	3.16 m.	3.21 v.s.
C	3.03 s.	3.0 v.w.	3.05 s.		3.02 s.	
I	2.93 m.	2.95 m.s.		2.93 v.w.		2.98 v.w.
I	2.60 s.	2.60 m.s.	2.60 m.s.	2.60 m.s.	2.60 m.	2.63 m.
I	2.42 w.	2.43 w.		2.41 ?	2.42 v.w.	
QC	2.28 w.	2.25 w.	2.30 w.		2.27 m.	
IC	2.10 w.		2.13 w.		2.10 w.	
I	2.01 v.w.	1.99 v.w.				
C	1.91 m.		1.92 w.			
C	1.88 w.					
Q		1.83 w.				
U	1.74 v.w.			1.75 v.w.	1.74 w.	
Q	1.69 v.w.	{ 1.70 w. 1.66	1.69 w-m.			1.70 w.
C	1.60 m.			1.60 v.w.		
Q	{ 1.54 m.	{ 1.53 m.s.	{ 1.54 m.			1.54 v.w.
C				{ 1.52 w.	1.52 m.	
I	{ 1.50 m.	{ 1.50 m.	{ 1.50 m.	{ 1.50 w.		
C	1.44 v.w.	1.45 w.				
C	1.41 v.w.					1.40 v.w.
Q	1.36 w.	1.38 w.				
IC	1.29 v.w.	1.30 w.				
C		1.25 w.				
I(C)	1.22 v.w.					1.24 v.w.
C	1.18 v.w.					
U	1.12 v.w.					

\* See footnote table 4.

certainty. Every sample of the colloids reported in this paper may, therefore, have contained small amounts of montmorillonitic clay.

It will be noted that the calcite reference pattern, as given in table 3, shows

a very strong 3.03Å. spacing, medium strong 1.92Å. and 1.87Å. spacings, and a number of other weaker spacings. The three strongest lines are the

TABLE 6b  
*Interplanar spacings for black-alkali-soil colloids*

MINER- ALS*	9		10	
	Air-dried	Heated to 500°	Air-dried	Heated to 500°
	Å.	Å.	Å.	Å.
I	10.0 w.	10.0 w-m.	10.0 w.	10.0 w.
U		5.1 w.		
I	4.5 v.s.	4.5 v.s.	4.5 m.s.	4.5 s.
Q	4.3 w.		4.2 w.	4.2 w.
CI		3.8 v.w.	3.8 w.	3.8 w-m.
IQ	3.35 s.	3.35 v.s.	3.35 v.s.	3.35 v.s.
I		3.20 m.s.		3.20 w.
C	3.05 v.s.		3.0 v.s.	3.05 w.
I		2.90 w.		
I	2.58 v.s.	2.60 s.	2.57 m.	2.57 m.
I	2.44 w.	2.42 w.	2.48 w.	2.43 w.
QC	2.28 m.	2.27 m.	2.27 s.	2.27 m.s.
IC	2.10 m.	2.18 v.w.	2.12 m.	2.12 m.
C			2.07 w.	
I	1.99 w.		{ 1.99 v.w.	1.98 w.
C	1.91 m.s.		{ 1.97 m.s.	
C	1.87 m.s.		1.90 m.s.	1.90 v.w.
Q	1.83 w.	1.81 w.	1.82 w.	1.82 m.
U	1.74 m.	1.73 w.		
Q	1.68 w.	1.67 w.	1.67 v.w.	1.67 m.
C	1.60 w.		1.60 v.w.	
Q	{ 1.54 m.	{ 1.54 m.	{ 1.54 w.	{ 1.54 m.
CI	{ 1.51 v.s.	{ 1.51 w.	{ 1.50 s.	{ 1.50
C	1.43 w.	1.46 w.	1.44 v.w.	
C			1.415 v.w.	
Q	1.38 w.	1.38 v.w.	1.38 m.	1.38 s.
IC	1.30 w.	1.30 w.	1.30 m.	1.29 m.
C	1.25 w.	1.26 w.	1.25 w.	1.26 w.
IC			1.23 w.	
U			1.20 w.	1.20 v.w.
C	1.18 v.w.		1.18 w.	1.18 w.
C	1.15 v.w.		1.15 w.	
U			1.08 w.	
C	1.04 v.w.		1.05 w.	
C	1.01 v.w.			

\* See footnote table 4.

most useful for identification purposes because of their intensity and also because they do not coincide with or even closely approach the position of any of the micallike clay or quartz lines. In agreement with the chemical analyses

and the dehydration curves, the strength of the  $3.00\text{\AA}$ . and  $1.92\text{\AA}$ . x-ray lines of the unheated colloids 1 and 2 indicates only small percentages of calcite. Only faint traces of other calcite lines are discernible in the films of these samples, and some of these were no doubt partly produced by other constituents, as indicated. On the other hand, samples 6 to 10 (tables 6a and 6b) gave strong or very strong  $3.00\text{\AA}$ . lines, some of them gave the  $1.92\text{\AA}$ . line, and two the  $1.87\text{\AA}$ . line. Other calcite lines, even a number of the rather weak ones, are also shown by these colloids. A very satisfactory correlation, therefore, was found between the x-ray data and the chemical analyses.

All seven of the black-alkali-soil colloids show the characteristic  $10\text{\AA}$ . line in either the heated or the unheated material. They also gave a spacing corresponding to the very strong  $2.56\text{\AA}$ . line, which ranks next in importance for the identification of the micalike clay. Although the presence of a  $10\text{\AA}$ . line is good evidence for the presence of micalike clays, the agreement was poor between other lines and the corresponding reference spacings in several of the samples, not only as regards the position of the lines but also in their relative intensities. Moreover, contrary to experience with authenticated muscovite and micalike clays, it was noted that heating the samples in some instances produced changes in the position of certain x-ray lines. The limited data at hand do not warrant an attempt to explain these variations. It would seem, however, that the micalike group of clay minerals probably includes a number of slightly different crystalline individuals having related structures but unit cells of somewhat different dimensions.

If it is assumed that micalike clay can be formed from parent rocks containing different kinds of micas, it is possible that the clays will show similar diversity. The x-ray lines of three different micas are shown in table 7. The spacings for muscovite were calculated on the basis of the unit cell of Jackson and West (7); those for biotite and lepidolite are based on the data of Mauguin (14) (see also 21, p. 351) after recalculation to an alternative cell equally consistent with his data but which lends itself to a closer comparison with muscovite. It will be noted that all of these micas show a general similarity but considerable variation in the position of some of their lines. The reference spacings for micalike clay (12, table 20) were taken from Grim, Bray, and Bradley (5) and are based on the assumption that this clay has a unit cell almost exactly like that of muscovite. These authors anticipated, however, that this clay type might be found to include a number of individual minerals similar in origin and general structure but varying in structural details. As suggested by the discussions of Nagelschmidt (15) and Hendricks and Alexander (6), it is still uncertain whether the micalike clay is merely an extremely finely divided form of mica or whether chemical and structural differences may not also be involved. From the viewpoint of origin alone, there appears to be room for a variety of micalike clays.

While, as already pointed out, the white-alkali-soil colloids failed to give any line corresponding to the strong  $3.21\text{\AA}$ . line of gedroizite, the black-alkali-

soil colloids all gave an x-ray line reasonably close to  $3.21\text{\AA}$ ., and this reflection was strong in the case of at least three of the samples. Although this line is given by the micalike clay and has been tentatively assigned to it, its intensity was greater than would be expected for micalike clay. The possibility, therefore, that the black-alkali-soil colloids contain some mineral similar to that described by Sedletzky, is not wholly excluded. But in view

TABLE 7

*Comparison of interplanar spacings having same Miller indexes for three mica structures*

MILLER INDEXES	MUSCOVITE	BIOTITE	LEPIDOLITE
	a = 5.18 b = 9.02 c = 20.04 $\beta = 95^{\circ}30'$	a = 5.30 b = 9.21 c = 20.16 $\beta = 95^{\circ}50'$	a = 5.20 b = 8.95 c = 19.82 $\beta = 94^{\circ}8'$
	$\text{\AA}$ .	$\text{\AA}$ .	$\text{\AA}$ .
(002)	9.98 s.	10.03	9.885
(004)	4.97 w.	5.015	4.94
(110)	4.47 s.	4.575	4.49
(022)	4.11 v.w.	4.285	4.075
(023)	3.70 v.w.	3.79	3.70
(114)	3.40 v.w.	3.54	3.43
(006)	3.31 m.	3.34	3.295
(114)	3.20 v.w.	3.24	3.22
(025)	2.98 w.	3.025	2.96
(115)	2.84 v.w.	2.89	2.88
(202)	2.56 v.s.	2.49	2.465
(133)	2.44 w.	2.51	2.44
(133)	2.38 m.	2.42	2.38
(22 $\bar{1}$ )	2.24 m.	2.30	2.245
(223)	2.18 w.	2.225	2.165
(043)	2.11 w.	2.18	2.12
(0010)	1.98 m.	2.01	1.98
(1, 3, 10)	1.65 w.	1.61	1.60
(312)	1.64 m.	1.67	1.65
(060)	1.50 s.	1.535	1.49
(335)	1.34 v.w.	1.39	1.37
(400)	1.29 m.	1.32	1.30
(0, 0, 16)	1.24 w.	1.25	1.24

of the uncertainties already noted, we are unwilling to accept these results as positive evidence for the presence of gedroizite in these colloids.

#### *Effect of heat on the decomposition of $\text{CaCO}_3$*

As pointed out already, heating the colloids to  $500^{\circ}$  either weakened the intensity of the calcite lines or obliterated them altogether. Since the conversion of  $\text{CaCO}_3$  into  $\text{CaO}$  ordinarily requires heating to a temperature somewhat above  $500^{\circ}\text{C}$ ., a few experiments were made to test the possibility that other substances present may have influenced the decomposition of calcite. Accordingly, precipitated  $\text{CaCO}_3$  and mixtures of the same with colloidal  $\text{SiO}_2$ ,

TABLE 8  
Effect of heating  $\text{CaCO}_3$  with other minerals

MINERALS*	$\text{CaCO}_3 + \text{HYDRATED SiO}_2$ 1:1		$\text{CaCO}_3 + \text{Fe(OH)}_2$ 1:1		$\text{CaCO}_3 + \text{MUSCOVITE}$ 1:8.8	
	Unheated	Heated to 500°	Unheated	Heated to 500°	Unheated	Heated to 500°
	Å.	Å.	Å.	Å.	Å.	Å.
I					ca 5.0 ?	4.97 w.
I					4.45 s.	4.45 s.
C	3.85 w.	3.85 w.	3.82 w.	3.70 m.	3.90 m.	3.87 w.
I					3.70 w.	3.70 w.
I					3.45 v.w.	3.42 w.
I					3.32 m.	3.31 m.
I					3.20 w.	3.18 w.
C	3.03 v.s.	3.03 v.s.	3.03 v.s.	3.03 v.s.	3.03 v.s.	3.00 m.
I					2.98 w.	
I					2.85 w.	2.85 w.
U					2.75 v.w.	2.75 w.
H				2.70 s.		
I					2.56 v.s.	2.56 v.s.
C	2.49 w-m.	2.49 w-m.	2.49 m.	2.50 w.	2.49 m.	
I					2.44 w.	
CaO (I)				2.40 m.	2.38 w.	2.38 s.
C	2.28 m.	2.28 m.	2.28 m.	2.28 m.	2.30 m.	2.27 m.
I						2.24
H				2.21 m.		
I					2.15 m.	2.15 m.
C	2.09 m.	2.09 m.	2.10 m.	2.10 w.	2.09 m.	2.07 w.
I					1.99 w.	1.99 w.
C	1.92 m.s.	1.92 m.s.	1.92 m.s.	1.92 m.	1.93 m.	1.92 w.
C	1.875 m.s.	1.875 m.s.	1.875 m.s.	1.88 m.		
H				1.84 m.		
CaO				1.70 s.		1.69 m.s.
I					1.65 w-m.	1.65 w-m.
C	1.62 v.w.	1.62 v.w.	1.61 w.	1.61 w.	1.61 w-m.	1.61 w.
	1.60 m.	1.60 m.				
I					1.56 v.w.	1.56 w-m.
C	1.52 w-m.	1.52 w-m.	1.52 w-m.	1.525 w.		
I					1.50 s.	1.50 s.
H				1.49 v.w.		
C	1.475 v.w.	1.47 v.w.	1.45 v.w.	1.455 v.w.	1.45 w.	1.44 w.
C	1.44 m.	1.435 m.	1.42 v.w.			
CaO						1.38 v.w.
U					1.36 v.w.	1.36 v.w.
C	1.34 v.w.	1.34 v.w.				
C(I)	1.30 w.	1.30 w.	1.30 v.w.	1.305 v.w.	1.30 m.s.	1.31 w.
I						1.29
H				1.26 v.w.		
C	1.25 v.v.w.	1.25 v.w.			1.25 w.	1.25 w.
C	1.23 w.	1.23 w.				
CaO						1.20 w.
C	1.18 w.	1.18 w.	1.175 v.w.		1.18 w.	
C	1.155 w-m.	1.155 w-m.	1.153 v.w.			
UH	1.145 v.w.	1.145 v.w.		1.14 v.w.		
H				1.105 v.w.		
CaO						1.07 w.
C	1.045 m.	1.045 w.	1.05 v.w.		1.05 w.	
C	1.013 w.	1.013 w.	1.012 v.w.			
CaO						0.98 v.w.
C	0.965 v.w.	0.965 v.w.	0.965 v.w.			
C		0.940 v.w.				

\* See footnote table 4. Additional symbols in this table: CaO = calcium oxide; H = hematite.

precipitated  $\text{Fe}(\text{OH})_3$ , or finely ground muscovite were x-rayed both before and after heating to  $500^\circ$ . The results reported in table 8 show that upon heating,  $\text{CaCO}_3$  was partly converted into crystalline  $\text{CaO}$ , particularly in the presence of  $\text{Fe}(\text{OH})_3$  or muscovite. It is interesting to note that, whereas the precipitated  $\text{Fe}(\text{OH})_3$  was entirely amorphous, the heated material gave several lines which agree with the theoretical spacings of hematite.

### *Solonetz colloids*

The colloids used in the foregoing part of this investigation were all taken from alkali soils which contain more or less soluble salts. Although some of them were relatively highly saturated with Na, none of the samples were taken from locations where the morphology of any horizon of the profile was typical of the solonetz as described by Russian soil scientists. On the contrary, they are saline soils with varying concentrations and composition of soluble salts. The term "solonetz" as used in this paper refers exclusively to a morphological and not to a chemical characteristic of the horizon in question. As was shown by Storie (20), Kelley (8), Kellogg (13), Rost (16), and Ellis and Caldwell (4), soil horizons with well-developed solonetz morphology are not necessarily high in either exchangeable Na or pH. In certain North American soils, the B horizon of which shows well-developed morphology closely similar to that of Russian solonetz, exchangeable Mg greatly exceeds exchangeable Na.

Since Sedletzky (19) reported that gedroizite is most abundant in the solonetz horizon of Russian alkali soils, it is of interest to examine the colloids of American soils which have well-developed solonetz morphology. With the exception of a few areas, this type of structure is not extensively developed in the United States, but in a number of localities solonetz morphology is found in limited areas. We have made an x-ray examination of the colloids of the solonetz horizon of the following soils:

11 [18216]. Antioch fine sandy loam, 19-24 inches, taken from Sec. 34, T 5 N, R 1 E,  $\frac{1}{4}$  mile southeast of Creed Station in Solano County, California. This horizon shows a well-developed columnar structure, is high in total clay, approximately neutral, and contains very little soluble salts of any kind. Mg constitutes about 60 per cent and Na only about 12 per cent of the total exchangeable cations.

12 [18345]. Antioch fine sandy loam, 18-24 inches, taken near Salinas, California. This horizon is columnar in structure and high in clay. It contains but little soluble salts, has a pH of 7.6, and is about 40 per cent Mg-saturated and 16 per cent Na-saturated.

13 [18348]. Huerhuero fine sandy loam, 17-28 inches, taken 5 miles east of Estrella School, San Luis Obispo County, California. This horizon is definitely columnar, high in clay, and contains very little soluble salts. Its pH is 7.07, and the soil is about 30 per cent Mg-saturated and 10 per cent Na-saturated.

The general chemical character of soils 11, 12, and 13 was reported by Kelley (8), and the character of the entire profile was fully discussed by Storie (20).

14 [18438]. Canby silty clay loam, 6-12 inches, taken  $\frac{1}{2}$  mile east of Canby, Modoc County, California. This horizon is a compact clay of columnar structure and contains slightly more soluble salts than samples 11, 12, and 13. It has a pH of 6.9 and is about 30 per cent Na-saturated and 20 per cent Mg-saturated.

TABLE 9  
*Interplanar spacings for the solonch colloids*

MIN- ERALS*	11		12		13		14		15	
	Air-dried Å.	Heated to 500° Å.	Air-dried Å.	Heated to 500° Å.	Air-dried Å.	Heated to 500° Å.	Air-dried Å.	Heated to 500° Å.	Air-dried Å.	Heated to 500° Å.
M	15.0 ?	10.0 ?	12.2 ?	10.0 w.					10.2 v.w.	10.2 v.w.
MI	7.0 v.w.		7.2 v.v.w.				7.0 v.v.w.			
K	5.5 w-m		5.5 w-m							
MU	4.4 s.	4.4 s.	4.4 s.	4.4 s.			4.4 w.	4.45 m.	4.5 v.s.	4.5 v.s.
MI							4.0 m.	4.05 m.		
QU			4.20 v.v.w.				3.7 v.w.	3.7 w.		
I									3.4 v.w.	3.39 w.
I(Q)	3.3 s.	3.32 s.	3.33 s.	3.33 s.	3.33 s.	3.30 s.	3.35 w.	3.35 w.		
Q	3.2 v.w.	3.18 w.	3.20 v.w.	3.20 w.	3.15 w.	3.14 w.	3.20 s.	3.20 s.	2.65 s.	2.64 s.
I										
I	2.55 m.s.	2.53 w.	2.57 w.	2.57 w.	2.57 m.s.	2.56 m.s.	2.57 m.	2.57 m.s.		
U							2.50 w-m.	2.50 m.		
I									2.45 w.	2.45 w.
I									2.20 v.w.	2.26 v.w.
I										2.18 w.
I										
I									2.00 v.v.w.	2.00 v.w.
Q	1.82 w.	1.80 w.	1.82 v.w.	1.82 v.w.	1.82 w.	1.82 w.			1.68 v.w.	1.68 v.w.
Q	{1.70 w.	{1.70 w.	{1.71 w-m.	{1.71 w.	{1.70 w-m.	{1.72 w.				
I	{1.65	{1.65	{1.66	{1.66	{1.66	{1.66				
I			1.54 w.	{1.54 w-m.					1.55 v.w.	1.55 m.
Q	1.50 s.	1.50 w.	1.50 s.	{1.50	1.50 s.	1.51 w-m.	1.50 m.	1.51 w.	1.50 v.w.	
I	1.37 w.	1.37 w.	1.37 w.	1.37 w.	1.37 w.	1.37 w.	1.37 w.	1.37 v.w.		
Q	1.29 w.	1.28 v.w.	1.29 v.w.	1.30 v.w.	1.28 v.w.	1.28 v.w.	1.29 v.w.	1.29 v.w.	1.30 w-m.	1.30 w.
I	1.24 w.	1.24 v.w.	1.24 v.w.	1.30 v.w.	1.24 v.w.	1.25 v.w.			1.25 v.w.	1.26 v.w.

\* See footnote table 4.



15<sup>a</sup>. Waukena fine sandy loam, 8-14 inches, taken  $\frac{3}{4}$  mile southwest of Buena Vista School, Tulare County, California. The upper part of this horizon has a well-developed columnar structure and the lower part a prismatic structure. It is low in soluble salts, has a pH of 9.3, and is relatively high in exchangeable Na. This sample probably more closely approaches the conditions referred to by Russian soil scientists as "solonetz" than any other sample reported herein.

The x-ray results are given in table 9. The patterns are similar to those of other soil colloids. Evidence is found for the presence of the three types of clay minerals which are common to soil colloids; namely, kaolinitic, montmorillonitic, and micalike clays. Quartz is also apparently present in all of the samples. The unidentified spacings are unusually few in number. The patterns were all rather weak on a darkened background, which suggests that considerable noncrystalline material was present.

The predominating clay mineral is of the micalike type as shown by the 10Å. and 2.56Å. spacings, which are especially characteristic of this group, as well as by other spacings of the micalike clays. Colloids 11 and 12 appeared to contain montmorillonitic and kaolinitic clays also, and colloids 13 and 14 gave indications of kaolinite. The montmorillonite lines were weak and uncertain, and at most only small amounts of this mineral could be present. The only definitely detectable clay mineral in colloid 15 is of the micalike type.

With the exception of sample 15, most of the spacings given by the solonetz colloids conform closely to those of micalike clay. In the pattern of colloid 15 the 10Å. line suggests micalike clay; but the other spacings agree less well with the reference pattern for this type. As has been pointed out already, the x-ray pattern of the micalike clays varies considerably and must be interpreted with caution.

Finally, the x-ray patterns of the five solonetz colloids afford little or no evidence for the presence of any mineral resembling gedroizite as described by Sedletzky. It is true, three of these colloids gave weak lines and one a strong line corresponding to the strong 3.21Å. spacing which Sedletzky considers to be characteristic of gedroizite. But, as previously indicated, the micalike clay pattern usually shows a weak 3.20Å. line, and it appears that until more precise criteria are established for the recognition of gedroizite, These data should not be interpreted as support for the presence of this substance. The lines characteristic of calcite were entirely absent from the patterns of the solonetz colloids.

#### DISCUSSION

It is especially interesting to note that both alkali and normal soils contain the same types of clay minerals. For example, in the several black-alkali soils reported herein, the Hanford and San Joaquin soils of California (12), and the Miami soil of the Mississippi Valley (1), micalike clays are prominent.

<sup>a</sup> Soil-survey sample, Visalia area, 5785-112.

On the other hand, the white-alkali soils of the Imperial Valley, the Yolo soils of California, the Putnam and certain other soil series of the Mississippi Valley, and certain European soil types, all contain montmorillonitic clay. As will be emphasized presently, however, it cannot be safely assumed that either white-alkali soils or black-alkali soils will always be found to contain the same kind of clay.

A high percentage of the clay minerals found in these alkali soils was probably contained in the alluvium when it was laid down in the areas sampled, rather than formed in the soils subsequent to the accumulation of soluble salts and the development of the existing alkali conditions. If reasoning based on other chemical processes is applicable to the formation of the clay minerals, then the initial formation of clay from the products of the weathering of primary silicates would not be expected to take place in alkali soils, except possibly to a limited extent, because the soluble products that are formed in this process cannot be leached out effectively. As is well known, alkali soils are soils of accumulation, not in the sense of horizontal accumulation of clay as in mature profile development, but the accumulation of soluble salts and also of partly weathered silicates in both the A and B horizons. Furthermore, but little evidence was obtained in this work indicating that the alkali conditions have produced any important change in the essential nature of the clays which were present in the original alluvium of these soils. The conclusion seems justified, therefore, that the effects of the alkali conditions have been largely limited to the replacement of ions and the dispersion of the clay, together with the accumulation of colloidal  $\text{SiO}_2$  and soluble salts.

If, on the other hand, the clay minerals of these alkali soils are truly pedological in origin, then the results are at variance with Sedletzky's hypothesis (17) as to the necessary conditions for the formation of montmorillonite, namely, alkaline weathering. As a matter of fact, this hypothesis is not supported by certain parts of the previous work of this laboratory (12) nor by that of Alexander, et al. (1). Both of the investigations referred to showed that the clay of certain soils of alkaline reaction is predominantly micalike rather than montmorillonitic. With some at least of those soils, however, it is, of course, possible that the clay minerals were also largely inherited from the parent materials and, therefore, the pH at which they were formed may have been unlike that of the soils. In the opinion of the authors, much more definite knowledge concerning the specific conditions required for the formation of the clay minerals will be obtained by the study of the residual weathering of primary minerals.

The foregoing discussion suggests that any type of clay that occurs within a given drainage basin might be found in the alkali soils of that basin regardless of the type and amount of alkali salts present. Further investigation is very likely to reveal, therefore, that white-alkali soils sometimes contain micalike or kaolinitic clay or both, as well as montmorillonitic clay; whereas

black-alkali soils will probably be found which contain kaolinitic, montmorillonitic, or micalike clay.

The results of this investigation are at variance with Sedletzky's results (18, 19) in that the clay of both the solonetz and the soda-containing soils was found to be predominantly micalike rather than gedroizitic in the one and montmorillonitic in the other. Moreover, one at least of the samples (no. 15) was separated from the solonetz horizon of a profile which closely resembles the published descriptions of the Russian solonetz. As pointed out already, the results of this investigation do not completely exclude gedroizite as a constituent of either the white-alkali or the black-alkali soils examined. But if gedroizite was present at all, the amount was certainly subordinate to that of micalike clays in the black-alkali soils and of montmorillonitic clay in the white-alkali types.

The fact that the removal of soluble substances by leaching is not active in alkali soils probably explains why colloidal  $\text{SiO}_2$  tends to accumulate in the upper horizons. It would indeed be surprising to find that  $\text{SiO}_2$  has been leached out when the much more soluble chlorides and sulfates have accumulated. Under the prevailing conditions, colloidal precipitation is to be expected, and these precipitates may contribute to some extent to the base-exchange properties of alkali soils.

The foregoing results show that fusion analysis of soil colloids cannot be relied on as definite indication of mineral types present. The principal reason why this is true is found in the extreme heterogeneity of soil colloids. Neither high  $\text{SiO}_2$  nor high ratio of  $\text{SiO}_2$  to  $\text{Al}_2\text{O}_3$  or  $\text{R}_2\text{O}_3$  is a definite indication of montmorillonite.

Finally, the thermal curves of soil colloids also require cautious interpretation, since chemical alteration involving changes in weight and heat may take place simultaneously with the loss of adsorbed water and crystal-lattice OH ions.

#### SUMMARY

The alkali-soil colloids investigated are characterized by relatively high  $\text{SiO}_2$  and CaO content. The latter was probably due in considerable part to the presence of  $\text{CaCO}_3$ . The  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios are also high in these samples, being more than 4.0 for all the samples analyzed. Their base-exchange capacities ranged from 25.7 to 58.4 m.e.

The white-alkali-soil colloids gave smooth wavelike dehydration curves which resemble those of Yolo soil colloids, whereas the curves for several of the black-alkali-soil colloids showed pronounced breaks between  $400^\circ$  and  $500^\circ\text{C}$ . This latter phenomenon has been definitely traced to the loss of  $\text{CO}_2$  from  $\text{CaCO}_3$ .

X-ray investigation showed that the white-alkali soils contain a mixture of montmorillonitic, kaolinitic, and micalike clays, whereas micalike clays greatly predominate in the black-alkali soils. The clay minerals of the horizon of

American soils showing the solonetz structure was also found to be chiefly micalike.

Considerable variation seems to be characteristic of the micalike clays. The suggestion is offered that this may be due in part at least to variations in the type of mica in the parent rocks from which the soil materials have been derived.

The clay minerals found in alluvial alkali soils have probably been inherited in considerable part as constituents of the alluvium and therefore are not necessarily pedological in origin. This view at once suggests that any type of clay mineral which occurs in a given drainage basin might be found in the alkali soils of that basin.

The x-ray results failed to support the idea that the base-exchange capacity of soil colloids is necessarily due to montmorillonitic clay. On the contrary, the sample with the highest base-exchange power gave virtually no x-ray evidence for the presence of montmorillonite.

The results of this investigation show that Sedletzky's conclusion regarding the formation of gedroizite is of doubtful applicability to American alkali soils. It is also doubtful whether pH is solely responsible for the formation of the several clay minerals found in soils. This, however, should not be interpreted to mean that pH is not important in the formation of the different clay minerals.

Apparently the effects produced on American alkali soils by the accumulation of soluble Na salts have consisted primarily in the exchange of cations together with the dispersion of the clay particles, but these processes probably have produced very little alteration in the essential structure of the clay minerals originally present. No convincing evidence was obtained that crystalline minerals peculiar to alkali soils have been synthesized in either the soda-containing type or the type which shows the solonetz morphology.

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# THE ABSORPTION OF PROTEINS BY MONTMORILLONITIC CLAYS AND ITS EFFECT ON BASE-EXCHANGE CAPACITY<sup>1</sup>

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The fact that organic substances have a tendency to react with inorganic clays has been demonstrated, but the exact mechanism of the reaction and the properties of the resulting complexes have not been fully investigated. Since the chemical, physical, and biological properties of a soil may be greatly influenced by the nature and content of organic matter, the relationship existing between the organic and inorganic fractions of soils deserves further study.

Ensminger and Gieseking (4) found that the absorption of proteins by montmorillonite is partly due to the cationic groups in the protein molecule. Since proteins are one of the most important nitrogenous substances added to soils, the present investigation was planned in order to study more fully the absorption of proteins by clays, using x-rays as a means of measuring the absorption, and to study the base-exchange properties of the resulting complexes.

## REVIEW OF THE LITERATURE

That various organic substances have a tendency to react with clays is evident from the investigations of Demolon and Barbier (2), Mattson (6), Myers (7), Gieseking (5), and Ensminger and Gieseking (4). Demolon and Barbier (2) found that humic colloids were more strongly absorbed by clays when the hydrogen-ion concentration of the system was high. According to Mattson (6) the isoelectric point of proteins is lowered by bentonite. He explained this change as being due to the formation of a nonionized compound between bentonite and proteins. Meyers (7) observed that the exchange capacity of a mixture of organic colloids and soil colloids was less than the sum of the capacities of the components. He also observed that the absorption of organic colloids was greatest in an acid suspension and suggested polar absorption as the most probable reaction involved. By x-ray analysis, Gieseking (5) found that complex organic cations were strongly absorbed within

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the variable (001) spacing of montmorillonitic clays. The replacement of inorganic cations by organic cations resulted in larger (001) spacings. Enslinger and Giesecking (4) found that gelatin and albumen were absorbed within the expansible portion of the montmorillonite crystal lattice, resulting in larger (001) spacings. These proteins were more strongly absorbed at low pH values. Furthermore, untreated gelatin was more completely absorbed than nitrous acid-treated gelatin, which indicates that the reaction was due partly to the presence of the free amino groups of the proteins.

#### EXPERIMENTAL TECHNIC

Wyoming bentonite (particles less than  $0.5\mu$  in diameter and predominantly montmorillonite) and Hartsburg colloid (particles less than  $1\mu$  in diameter) were used throughout this investigation. The Hartsburg colloid was isolated from the A-horizon of Hartsburg silt loam, which is an imperfectly developed grassland soil derived from deep loess on nearly level to depressional topography, in association with Muscatine silt loam.

The first step in the preparation of the protein-clay complexes was the mixing of an alkaline suspension of clay with an alkaline suspension of protein. Under these conditions both the clay and protein are negatively charged, and thus a homogeneous mixture is easily obtained. The mixture was then acidified to the desired pH by slowly adding dilute acetic acid. It was necessary to follow the dilute acetic acid by concentrated acetic acid where low pH values were desired. The acidified complexes were shaken in a mechanical shaker for 48 hours. Since microbial activity was rather great at certain pH levels, a few drops of toluol were added to prevent microbial decomposition. The complexes used for diffraction studies were filtered, washed with water, and dried over anhydrous magnesium perchlorate. Base-exchange studies were made on both dried and moist complexes.

The base-exchange capacity was determined by the barium method. The excess acid was washed out of the complex with water, and then the complex was saturated with barium by leaching with barium chloride solution. The unabsorbed barium was removed by leaching with double-distilled water until the leachate no longer gave a test for chloride. The absorbed barium was replaced by washing with 0.1 *N* HCl and the barium determined gravimetrically.

#### EXPERIMENTAL RESULTS

##### *Absorption of proteins by montmorillonite*

The (001) spacings of montmorillonite treated with varying quantities of hemoglobin, casein, protamine, pepsin, and pancreatin are reported in table 1. The dried samples were placed in a closed system and connected to a magnesium perchlorate desiccator while the x-ray diffraction studies were being made.

*Absorption of gelatin by Hartsburg colloid*

Since the Hartsburg colloid contained 15 to 20 per cent montmorillonitic type of clay, it was possible to measure the amount of absorption by determining the size of the (001) spacings. The effect of increasing amounts of gelatin on the (001) spacings of the montmorillonitic fraction of Hartsburg clay is given in table 2.

TABLE 1  
*Absorption of proteins by montmorillonite*

PROTEIN ADDED	QUANTITY OF PROTEIN ADDED TO 1 GM. MONTMORILLONITE	(001) SPACINGS OF MONTMORILLONITE TREATED WITH PROTEINS
	gm.	Å.
Hemoglobin.....	0.125	13.5
Hemoglobin.....	0.25	15.0
Hemoglobin.....	0.50	23.0
Hemoglobin.....	0.75	25.0
Hemoglobin.....	1.00	26.9
Hemoglobin.....	1.25	29.8
Casein.....	1.00	41.5
Casein.....	2.00	48.0
Protamine.....	1.00	17.5
Pepsin.....	2.00	17.5
Pancreatin.....	2.00	17.5

TABLE 2  
*Absorption of gelatin by Hartsburg colloid*

QUANTITY OF GELATIN ADDED TO 1 GM. HARTSBURG CLAY	(001) SPACINGS OF MONTMORILLONITE IN HARTSBURG CLAY
gm.	Å.
0.125	15.0
0.25	17.5
0.50	17.5
1.00	17.5

*Effect of gelatin absorption on the base-exchange capacity of montmorillonite and Hartsburg colloids*

Figure 1 shows that gelatin has a very marked effect on the exchange capacity of montmorillonite and Hartsburg colloids. These gelatin-clay complexes were prepared by mixing alkaline suspensions of gelatin and clay together and then acidifying to pH 2.6 with acetic acid.

*Effect of pH on base-exchange capacity of montmorillonite-protein complexes*

The data in figure 1 show that proteins decrease the base-exchange capacity of montmorillonite at low pH values. Since most soils have a pH value above



5.0, it would be of value to know the effect of protein absorption over a wide range of pH values. The data presented in table 3 show that gelatin decreases the base-exchange capacity of montmorillonite materially even at pH values above 5.0.

*Effect of treated proteins on exchange capacity of montmorillonite*

In order to learn more about the type of reaction taking place between proteins and clays, proteins were treated so as to change their basic properties.

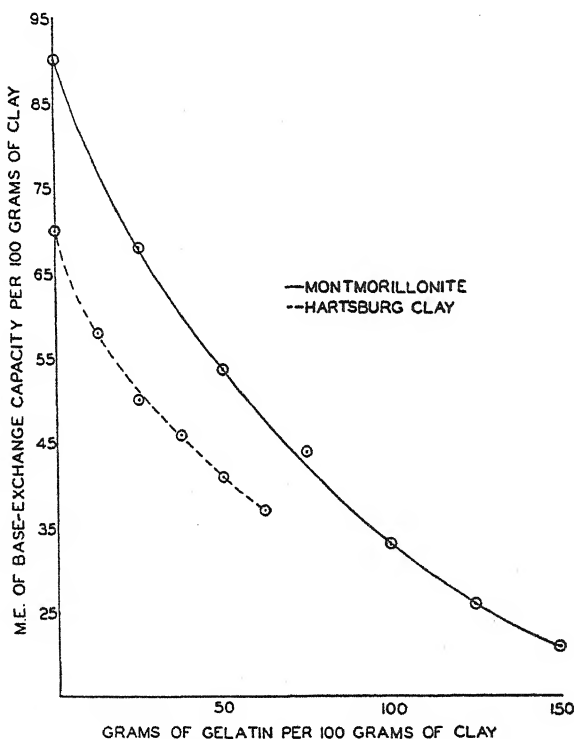


FIG. 1. EFFECT OF GELATIN ABSORPTION ON THE BASE-EXCHANGE CAPACITY OF MONTMORILLONITE AND HARTSBURG COLLOIDS

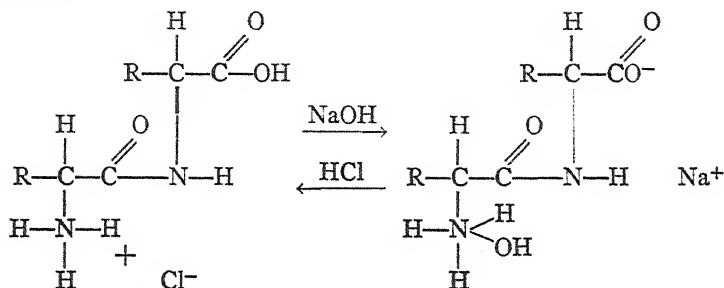
There are two ways of altering the basic or cationic properties of proteins; namely, by formaldehyde treatment and by nitrous acid treatment. These treatments were applied to albumen, gelatin, and hemoglobin. The samples obtained in this manner were then used in absorption experiments with montmorillonite. According to the data in table 4, formaldehyde-treated hemoglobin did not reduce the exchange capacity as much as did untreated hemoglobin. Formaldehyde-treated gelatin and albumen gave the same result as untreated.

Since nitrous acid-treated albumen and hemoglobin did not reduce the

exchange capacity of montmorillonite, evidently all the basic groups had been destroyed. On the other hand, nitrous acid-treated gelatin must have still contained basic groups, for it reduced the base-exchange capacity materially.

#### DISCUSSION OF RESULTS

The amphoteric nature of proteins must be kept clearly in mind when discussing the data presented. This may be represented by the following equilibrium:



The addition of an acid increases the ionization of the amino group and decreases the ionization of the carboxyl group. Therefore, an increase in hydrogen-ion concentration causes the protein molecule to act more like a cation. Proteins are electrically neutral at the isoelectric point, but this should not be taken to mean that they are electrically inactive as far as absorption is concerned. Above the isoelectric point they are predominantly negatively charged, but again they possess some basic properties and are absorbed to a certain extent by negatively charged clays. Egg albumin, gelatin, and hemoglobin have the following isoelectric points: 4.6–5.0, 4.4–5.6, and 6.8, respectively. Any chemical treatment which would alter the basic or acidic groups of proteins would also affect their isoelectric points. The treatment of gelatin with formaldehyde shifted the isoelectric point from pH 4.75 to pH 4.3. Deaminized gelatin had an isoelectric point of 4.0 as compared with 4.7 for untreated gelatin.

#### *Absorption of proteins by montmorillonitic clays*

The data presented in this paper substantiate the theory of Engsminger and Gieseck (4) that proteins are absorbed as cations within the (001) spacings of montmorillonite. Albumen was found to be absorbed from alkaline solution, as was gelatin in previous work. It did not seem, however, to be so strongly absorbed under alkaline conditions as did gelatin.

Hartsburg colloid contains a montmorillonitic clay which absorbs large ions within the (001) spacing. The (001) spacing of Hartsburg was not so expandable as the (001) spacing of montmorillonite. The largest spacing obtained was 17.5Å., regardless of the amount or nature of the organic substance

added. This lack of expansibility may be due to less base-exchange capacity within the (001) spacing or possibly to a reduction in exchange capacity as a result of previous absorption of positively charged hydrated sesquioxides or organic matter.

TABLE 3

*Effect of hydrogen-ion concentration on the base-exchange capacity of montmorillonite-gelatin complexes*

FINAL pH OF SUSPENSION DURING PREPARATION	BASE-EXCHANGE CAPACITY OF COMPLEX*
	<i>m.e.†</i>
9.00	90.0
7.6	85.0
6.5	73.4
5.3	59.7
3.45	31.1
2.00	23.1

\* 1 gm. gelatin to 1 gm. montmorillonite.

† Per 100 gm. montmorillonite.

TABLE 4

*Effect of treatment on the base-exchange capacity of montmorillonite-protein complexes*

AMOUNT AND KIND OF PROTEIN ADDED TO 1 GM. MONTMORILLONITE	pH	BASE-EX- CHANGE CAPACITY	TREATMENT OF COMPLEX OR PROTEIN BEFORE DETERMINING BASE-EXCHANGE CAPACITY
		<i>m.e.*</i>	
1 gm. gelatin .....	9.0	82.5	Dried the complex
1 gm. gelatin .....	7.0	74.5	Dried the complex
1 gm. gelatin .....	4.5	35.6	Dried the complex
0.25 gm. gelatin .....	3.0	70.5	H <sub>2</sub> C=O treated gelatin
0.25 gm. albumen .....	3.0	71.0	H <sub>2</sub> C=O treated albumen
0.25 gm. hemoglobin .....	3.0	70.0	H <sub>2</sub> C=O treated hemoglobin
0.25 gm. gelatin .....	3.0	75.0	HNO <sub>3</sub> treated gelatin
0.25 gm. albumen .....	3.0	93.0	HNO <sub>3</sub> treated albumen
0.25 gm. hemoglobin .....	3.0	91.5	HNO <sub>3</sub> treated hemoglobin
0.25 gm. albumen .....	3.0	71.0	None
0.25 gm. hemoglobin .....	3.0	56.0	None

\* Per 100 gm. clay.

#### *Base-exchange studies on protein-clay complexes*

Since proteins are absorbed by montmorillonitic clays, they offer a good means of studying further the effect of organic material on the base-exchange capacity of clays. Gelatin was used in most cases because it is soluble under the conditions of this experiment, and it forms a clay complex which filters readily.

Saturation with barium is a very satisfactory method of determining the

base-exchange capacity of these complexes. According to Docking and Heymann (3) gelatin treated with barium chloride and washed free of chloride ions increased in ash content 0.13 per cent. On the basis of 100 gm. of gelatin, this increase would amount to only 1.7 m.e. It is assumed, therefore, that virtually all the barium absorption was due to the clay fraction of the complex.

The data presented show that absorbed proteins have a marked effect on the base-exchange capacity of clays. The amount and nature of the protein added determine the magnitude of the reduction in base-exchange capacity. Proteins did not reduce the base-exchange capacity of clays in an alkaline medium. As the hydrogen-ion concentration is increased, however, the base-exchange capacity decreases. The fact that an increase in hydrogen-ion concentration increases the basic properties of proteins would indicate that these are absorbed as cations. Many soils have a pH value low enough to induce considerable reaction between the organic and the inorganic fractions. Moreover, neutral soils may have acid zones due to carbon dioxide from roots and decomposing organic matter which would cause a chemical reaction between the organic and the inorganic fractions.

Treatments which alter the basic properties of proteins cause them to be less absorbed. The fact that nitrous acid-treated albumen and hemoglobin caused no decrease in exchange capacity whereas the untreated proteins caused a marked decrease is further evidence that they were absorbed before treatment as a result of basic amino groups. Nitrous acid-treated gelatin decreased the exchange capacity. This may be explained by the work of Bancroft and Barnett (1), who found that deaminized gelatin had a combining capacity for hydrochloric acid amounting to 0.0044 gm. equivalent. They suggested that the combining capacity of deaminized gelatin is due to the

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groups which are not attacked by nitrous acid.

Many investigators agree that the addition of organic matter to clays reduces the base-exchange capacity of the latter. The mechanism involved, however, has not been agreed upon. Sideri (8) suggested selective orientation as the reason for the reaction between clay and humus. Myers (7) reported a reduction in exchange capacity of clays when mixed with organic colloids and explained it on the basis of polar absorption. According to Myers this polar absorption should result in a close packing of organic colloidal particles on the surface of the clay. Such packing would result in a reduction in the exchange capacity not because of a chemical reaction but because of steric hindrance to the passage of ions from the ends and sides of the organic colloids. He expressed the belief that absorption would probably be a factor in reducing the exchange capacity of inorganic colloids by covering the exchangeable ions on the surface of the clay and thus permitting only the ions within the expandable lattice to be exchangeable. X-ray diffraction data show, however, that proteins also enter the expandable portion of the montmorillonite lattice.

On the basis of the data presented here a chemical reaction between the basic groups of the proteins and the negative charge on the clay seems to be the most probable type of reaction. Treatments which increase the basic properties of proteins increase the absorption of proteins by clays, as revealed by x-ray diffraction data. Treatments which decrease the basic properties result in less absorption and a smaller decrease in the exchange capacity of the clay.

#### CONCLUSIONS

The x-ray data presented here show that proteins were absorbed within the expansible portion of the lattice of montmorillonitic clays. The change in the (001) spacing of montmorillonite resulting from the absorption of proteins depended on the amount and nature of the protein added. The montmorillonitic clay in Hartsburg colloid was not found to be so expansible as montmorillonite from bentonite.

Proteins reduced the base-exchange capacity of montmorillonite and Hartsburg clay materially when the complexes were acidified. No reduction occurred in an alkaline suspension, however.

Such treatments as formaldehyde and nitrous acid which alter the basic properties of proteins resulted in less absorption. An increase in hydrogen-ion concentration resulted in greater absorption due to greater ionization of the amino groups. These data would indicate that proteins are partly absorbed as cations.

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# UNRELIABILITY OF THE BENZIDINE COLOR REACTION AS A TEST FOR MONTMORILLONITE

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Hendricks and Alexander (3) recently asserted that, in the absence of organic matter, benzidine or its hydrochloride gives a blue color with montmorillonite, that the reaction is specific enough to characterize this mineral "in the presence of other clay minerals and constituents of soils," and that the test has been used "on a number of clay fractions from soils and has been found to be completely trustworthy in the absence of organic matter." Hauser and Leggett (2) observed a series of color reactions between various amines and different clays. They found that "the color is specific to the amine and can be produced with all reactive types of clay (bentonite, fuller's earth, kaolin, zeogel, etc.)." Since this laboratory is engaged in the investigation of the minerals of soil colloids, the color reaction with benzidine hydrochloride has been rigorously examined to determine its nature and suitability as a test for montmorillonite.

## TESTS WITH VARIOUS MATERIALS

The following tests were made in accordance with the directions given by Hendricks and Alexander, using a saturated aqueous solution of benzidine hydrochloride (after oxidation of organic matter with  $H_2O_2$  where necessary):

1. Ten colloids from alkali soils which have recently been thoroughly examined in this laboratory (5) were tested. The results indicate that only samples 1, 3, 4, and 5 contain enough montmorillonite to be detected by the x-ray method of analysis; the remaining six colloids apparently are micalike clays. The results of the tests are presented in table 1.

These results, though not necessarily in conflict with those reported by Hendricks and Alexander, suggested, nevertheless, that some factor other than, or in addition to, montmorillonite may have contributed to the production of the color. This was indicated by the fact that the intensity of the blue color developed by these colloids was not proportional to the amount of montmorillonite present as revealed by x-ray analysis. Neither was it proportional to the amount of micalike clay in the samples.

2. Soil colloids the mineral nature of which has been reported in a previous

<sup>1</sup> The author expresses appreciation to W. P. Kelley for advice and assistance in the preparation of the manuscript and to W. H. Dore for making the x-ray determinations.

paper from this laboratory (4) were tested. The results are presented in table 2.

These results are not in agreement with Hendricks and Alexanders' assertion that "kaolinitic clay minerals and other inorganic constituents of soils give negative results." Examination of the x-ray data on these colloids (4) reveals no indication of montmorillonite in samples 18882, 18883, 18885, 18886, and 18887, and only slight indication in sample 16307, yet all these samples gave moderate to strong color reactions. Furthermore, the last-named sample probably contains much less montmorillonite than does Yolo colloid 17559, yet these two colloids gave approximately equal color tests with benzidine.<sup>2</sup> Hendricks and Alexander also found exceptions, observing that the Miami and desert soils (micalike clay types) gave the color test. In explanation, they suggest that micalike clay often "grades into a mixed layer mineral with montmorillonite as a component." As will be shown later, there is an equally

TABLE 1  
*Colorimetric tests on colloids from various alkali soils*

SAMPLE NUMBER	SOURCE	COLOR INTENSITY
1	Fresno, California	Moderate
2	Fresno, California	Moderate
3	Imperial Valley, California	Strong
4	Imperial Valley, California	Strong
5	Imperial Valley, California	Very strong
6	Lake Rosamond, California	Very weak
7	Fallon, Nevada	Very strong
8	Vale, Oregon	Weak
9	Phoenix, Arizona	Moderate
10	Corinne, Utah	Very weak

plausible alternate explanation. It can be noted that in general the trend is toward stronger tests for the montmorillonite type samples, but the numerous exceptions seem to indicate that the test cannot be relied upon.

3. A series of bentonites were tested, all of which have been shown by x-ray studies to be high in montmorillonite. These materials were first tested in their crude state by allowing a piece of the rock to hydrate and swell in distilled water overnight, after which the benzidine hydrochloride was added. The results are given in table 3.

With most of these samples the color was developed in streaks and spots

<sup>2</sup> Modern methods of mineralogical analysis of clays are not sufficiently sensitive to preclude the possibility that there may be small amounts of montmorillonite in materials of this kind. The color intensity observed in the micalike and kaolinitic samples, however, was entirely disproportionate to the amount of montmorillonite which could have been present. For this reason it is believed that the color observed can best be explained as due to the presence of some substance other than montmorillonite.

scattered throughout the material, indicating heterogeneity with respect to either montmorillonite or some other factor which might influence color development. Failure of some of these montmorillonitic samples to give a

TABLE 2  
*Colorimetric tests on colloids from different California soils*

SAMPLE NUMBER	SOIL SERIES	DEPTH <i>inches</i>	PREDOMINATING MINERAL TYPE	COLOR INTENSITY
7083	Yolo	0- 8	Montmorillonite	Moderate
17399	Maxwell	0-18	Montmorillonite	Strong
17557	Yolo	0- 8	Montmorillonite	Very strong
17558	Yolo	9-12	Montmorillonite	Very strong
17559	Yolo	25-48	Montmorillonite	Strong
18882	San Joaquin	0-20	Micalike	<i>Moderate</i>
18883	San Joaquin	22-30	Micalike	<i>Strong</i>
18885	San Joaquin	24-32	Micalike	<i>Strong</i>
18886	Hanford	0-30	Micalike	<i>Moderate</i>
18887	Hanford	0-24	Micalike	<i>Moderate</i>
16054	Vina	0-12	Kaolinitic	Weak
16056	Vina	0-12	Kaolinitic	Weak
16057	Keefers	0-10	Kaolinitic	Very weak
16303	Redding	1-15	Kaolinitic	Weak
16307	Redding	20-37	Kaolinitic	<i>Strong</i>

TABLE 3  
*Colorimetric tests on various bentonites*

SAMPLE NUMBER	SOURCE	COLOR INTENSITY
2	Otay, California	Very strong
3	Clay Spur, Wyoming	Strong
4	Merritt, B. C., Canada	Strong
5	Rosedale, Alberta, Canada	Very strong
6	Princeton, B. C., Canada	Strong
7	Goldfield, Nevada	Very weak
8	Medicine Bow, Wyoming	Very strong
12	Vidal, Riverside Co., California	Weak
15	Death Valley, California	No color
..	Highbridge, Kentucky (Ordovician bentonite)	Weak
..	Smith Co., Mississippi	Strong

positive test suggests that the necessary factor may have been absent. Hendricks and Alexander also reported that certain samples of montmorillonite gave only weak tests.

A series of colloids separated by sedimentation from the samples of crude bentonite and saturated with various cations was tested. One sample of ben-



tonite 2 gave but a very weak test with benzidine, whereas another from the same deposit gave a strong test. The colloid separated from bentonite 3 failed completely to give the test, even though the crude material gave a strong color. Since these colloids are known to be montmorillonitic, the results indicate that montmorillonite alone is insufficient to give the color, and that the factor responsible for color development may be readily separable from the colloid (the colloids were separated by gravity sedimentation and had been subjected to no drastic treatment).

No effect of the adsorbed cation was observed except in the case of easily reducible cations. Portions of colloid 7, each saturated with different cations (Ca, Ba, Mg, K, Na, and  $\text{NH}_4$ ) all gave weak tests, yet a portion of this colloid saturated with  $\text{Cu}^{++}$  gave a strong test; this point will be referred to later. Other materials may also give the test: for example, a commercial permutite gave a moderate color reaction, and a commercial sample of kaolin and a supposedly pure sample of hydrated halloysite both gave moderate tests, although the best obtainable sample of kaolinite failed to give the reaction.

The results obtained suggested that some factor other than montmorillonite was active in producing the color and that this factor is not always associated exclusively with montmorillonite.

#### CONSIDERATIONS ON THE REASONS FOR COLOR DEVELOPMENT

As was pointed out by Hendricks and Alexander, the color developed is undoubtedly due to the formation of a semiquinone compound resulting from the partial oxidation of the diamine. These authors suggest that iron may be connected in some way with the color development. They point out: "It is probable that the oxidation is due to the presence of ferric iron or an equivalent oxidizing agent in an insoluble form together with or a part of the montmorillonite." They further suggest that the failure of pure samples of montmorillonite to give the color is "due apparently to the absence of iron compounds." This idea was not developed further, except for the observation that "freshly prepared ferric hydroxide and the iron compounds present in soils did not give semiquinone in the absence of montmorillonite." Hendricks and Alexander also pointed out that after the addition of codeine or brucine to a sample of strongly reactive montmorillonite, the sample gave only a faint test. They concluded "that the large and more basic alkaloid molecule is far more firmly held as a cation than is benzidine and thus prevents formation of the latter salt. This experiment also demonstrated that the presence of an oxidizing agent alone in the clay is inadequate for the test." They seem to conclude, therefore, that the formation of a benzidine-montmorillonite salt and the presence of an available oxidizing agent are essential to the test.

Hauser and Leggett (2) concluded that the color was not produced by the action of an oxidizing agent "adsorbed or entrapped in the clay structure." Instead, they suggested that the clay particle itself can act as the oxidant, accepting an electron from the adsorbed amine. This causes the production

of the colored compound through "setting up an unbalanced force field in the amine" which "provokes resonance in the amine and the quinoidal structure is obtained."

Consideration of the structure of montmorillonite and search of the literature on the semiquinones revealed nothing which would indicate a possible role of montmorillonite in the production of these compounds. Michaelis (6) gives an excellent summary of the knowledge of the semiquinones. Some of the essential points in his paper are, briefly, as follows:

The oxidation to semiquinones is unique in organic chemistry in that it involves the transfer of but a single electron (corresponding roughly to  $\text{Fe}^{++} \rightarrow \text{Fe}^{+++}$ ). The resulting compound is monomolecular, the odd electron effectively oscillating between the two amine groups of the partly oxidized molecule. The semiquinone is strongly colored and may or may not develop another colored compound upon further oxidation. The formation and existence of these compounds is dependent upon pH and the oxidation potential of the system. The benzidine compounds are very labile—so much so that these compounds are difficult to work with. Even the dry compounds are somewhat unstable and are very readily oxidized. In moderately acid solutions a two-step oxidation occurs, causing production of the brown colored oxidation product, the semiquinone being unstable in this range.

From these considerations it seems possible that the color developed when benzidine is added to certain soil colloids or mineral types results from the action of some available oxidizing agent in the material and that the role of montmorillonite is purely incidental and not essential to the reaction.

#### EXPERIMENTS ON COLOR DEVELOPMENT

It was found that soluble ferric salts, even when present in very low concentrations, produce a very strong blue color with benzidine. This color is readily produced by  $\text{FeCl}_3$  in the total absence of montmorillonite or any other mineral, as would be expected from the mode of formation of semiquinone compounds. As was found by Hendricks and Alexander,  $\text{Fe}(\text{OH})_3$  appears not to give the test. This is apparently due to the great insolubility of this compound. If, however, a large amount of the weakly acidic benzidine hydrochloride solution is added to but a little  $\text{Fe}(\text{OH})_3$ , a faint blue color is slowly produced, suggesting that the solubility explanation is correct. Since  $\text{Fe}^{+++}$  alone is able to produce the color, it is suggested that the color, produced with many soil colloids and minerals, results from the oxidizing action of ferric iron present in the material, probably in most cases as an impurity.

#### TESTS ON THE ROLE OF IRON IN COLOR DEVELOPMENT

The aforementioned alkali soil colloids were subjected to the modified Truog treatment (7) using nascent  $\text{H}_2\text{S}$  to remove free iron compounds. Although all these materials originally gave a positive test with benzidine (table 1), no color was produced by any of them subsequent to the treatment even after standing several days. Similar results were obtained on five different bentonites, both the crude material and the separated colloids, and on the Yolo

soil colloid (tables 2 and 3) after extraction with a nonreducing iron solvent, namely, oxalic acid dissolved in sodium oxalate as proposed by Drosdoff (1). In every case, removal of the more soluble iron compounds resulted in the destruction of the power to produce the color. This was true regardless of the mineral nature of the colloid. X-ray studies showed that the essential structure of the clay minerals was not altered by the acid treatments. Fusion analysis, however, showed that these extracted materials still contained considerable ferric iron. The same was found with colloids from bentonites 2 and 3, which normally give little or no color. This agrees with reasonable expectation, since iron occurring as a lattice constituent would not be expected to effect the oxidation unless it were exposed on a broken edge. Grinding in a porcelain mill with agate balls caused these unreactive iron-containing materials to give a strong test, suggesting that the ferric iron was present in the interior of the crystals, either occluded or as a lattice ion, and was thus unavailable for chemical action until exposed by grinding.

Traces of  $\text{FeCl}_3$  were added to montmorillonitic colloids after they were made nonreactive by acid extraction. In every case a blue color developed immediately, which upon shaking became adsorbed by the colloid, thus giving every appearance of positive tests again. This type of experiment was repeated on other materials which normally give little or no color with benzidine. The following materials were tested: colloid from bentonite 3, two samples of kaolinite, a commercial permutite, and three samples of halloysite. Benzidine was first added, then a trace of  $\text{FeCl}_3$ . With each of the materials blue color was immediately developed in the solution upon addition of  $\text{FeCl}_3$ . After shaking, the colored compound was adsorbed by the material, giving the appearance of a positive test for montmorillonite. If sufficient colloidal material was present, the supernatant liquid became completely colorless, while the flocculated colloid was deeply colored. The only difference discernible between these materials was in their relative decolorizing power, which appears to be a function of the adsorptive power of the material.

By saturating nonreactive colloid from bentonite 3 with benzidine and then adding  $\text{FeCl}_3$  to the colorless material, it was found that the benzidine could be oxidized to the semiquinone while adsorbed. By reversing the procedure, that is saturating with  $\text{Fe}^{+++}$  then adding benzidine, it was found that adsorbed  $\text{Fe}^{+++}$  can also effect the oxidation. This explains the effect with  $\text{Cu}^{++}$  noted previously, since  $\text{Cu}^{++}$  can act as an oxidizing agent for benzidine. Other materials, such as kaolinite and halloysite, were found to respond in the same way. Hence no evidence was obtained indicating that montmorillonite is essential for the color development or that it plays any unique role. It does, however, have a slightly greater efficiency as an adsorbing agent.

It is not intended to suggest that iron is the only material which can effect the oxidation of benzidine. In testing other oxidizing agents, it was found that benzidine is very easily oxidized by  $\text{H}_2\text{O}_2$ ,  $\text{MnO}_4^{--}$ ,  $\text{Cr}_2\text{O}_7^{--}$ ,  $\text{Br}_2$  (bromine water),  $\text{IO}_3^-$ ,  $\text{HNO}_3$ ,  $\text{Fe}^{+++}$ ,  $\text{Fe}(\text{CN})^{--}$ , and  $\text{Cu}^{++}$ . A milky white precipitate is formed with  $\text{Fe}^{++}$  and  $\text{SO}_4^{--}$ . Apparently the blue semiquinone is

stable only in a limited range of pH on the acid side of neutrality, the brown, fully oxidized compound being produced by oxidation in stronger acid solutions. A yellowish brown is obtained in strongly alkaline solutions. Strong oxidizing agents tend to convert the material over to the brown, fully oxidized product, but with care the blue intermediate product may be obtained. In reaction ranges in which the semiquinone is stable, all the aforementioned oxidizing agents convert the material to the brown compound. Careful addition of base to acid solutions in which the brown color has been developed causes production of yellow, green, blue, and then again brown solutions.

With the demonstrated ease of oxidation of benzidine, it is apparent that mere traces of iron, manganese, or other reducible material in the soil should be sufficient to produce the color. It is usually thought that the iron compounds associated with soil colloids are relatively insoluble. The pH of the saturated benzidine hydrochloride solution used in making the tests was 2.3, however, and it is believed that this reaction is sufficiently acid to bring enough iron into solution for the test.

This possibility was explored by adding a few crystals of KSCN to aqueous suspensions of the colloids, after which the tubes were allowed to stand for 1 minute without shaking. A test indicating water-soluble  $\text{Fe}^{+++}$  was obtained in nearly every case in which a positive test with benzidine had been obtained. If sufficient dilute HCl was added to bring the suspension to the same reaction as that of the benzidine-colloid suspension (usually about pH 3.5), a test for  $\text{Fe}^{+++}$  was obtained with every material which had previously given the blue color with benzidine. It was further observed that no test for  $\text{Fe}^{+++}$  was obtained with materials which failed to give the benzidine test, and that with the soil colloids (tables 1 and 2) there was a very close parallel between the relative intensity of color obtained with KSCN and with benzidine.

#### DISCUSSION AND CONCLUSIONS

Since benzidine can be readily converted into a blue-colored semiquinone by the action of oxidizing agents, and montmorillonite is neither necessary for nor sufficient in itself to give the color test, and since, with proper oxidizing conditions, other mineral types give the same effect as montmorillonite, it is felt that the benzidine test cannot be considered as specific for this mineral.

The facts that the presence of slightly soluble iron was demonstrated in materials which gave the test, and that removal of the more soluble iron compounds caused loss of the power to give the test, are taken as rather strong indication that iron compounds, probably present as impurities in the samples, were, in most cases, responsible for production of the color reaction.

The actual iron compounds which might contribute to the color development are, of course, difficult to determine. Apparently several or all of the usual hydrated oxides of iron, which occur as impurities coating soil colloids or in certain rock and mineral samples, are sufficiently soluble to yield  $\text{Fe}^{+++}$  under the conditions of the test. It has been shown that adsorbed  $\text{Fe}^{+++}$  can produce the color, and it would certainly be expected that such iron might also occur

in soil colloids. The results have also shown that materials may contain considerable  $\text{Fe}^{+++}$  yet be unable to give the test, suggesting that either the iron was present occluded within crystal aggregates and hence not available to chemical action, or that it was a constituent of the lattice. In the latter case it should also be effectively unavailable. If, however, crystal lattice  $\text{Fe}^{+++}$  is exposed on broken crystal edges, it may be capable of reduction. In this case the iron should behave similarly to adsorbed iron and hence contribute to color development. This latter type of iron may in a large measure account for the greater tendency of montmorillonite and mica-like clay samples to give the test, since these minerals probably contain iron as a common lattice constituent, whereas such iron is unknown in the kaolinitic type of clay materials.

Manganese and other reducible materials in soils should also influence color development, but it is believed that iron is by far the most important.

In the light of the chemistry of the semiquinones (6) and the results obtained above, it is believed that the color developed between clays and certain diamines can best be explained as due to direct oxidation to the semiquinone compound as a result of the action of some oxidizing agent present, usually as an impurity in the clay. Further, since it was demonstrated that montmorillonite is not essential to the reaction, that the proper oxidizing conditions though sometimes present are not always associated with montmorillonite, and that the oxidizing agents may also be associated with other clay constituents of soils, it is concluded that this test cannot be considered as specific for montmorillonite. Instead, the color reaction is thought to be indicative merely of the presence of some oxidizing impurity in the material tested. The data obtained are in essential agreement with those of Hauser and Leggett, in that the oxidation of benzidine can be produced in the presence of various kinds of clays and also that pH has a marked effect on the oxidation.

It is thought, however, that the explanation offered above more nearly explains the process than their suggestion that the clay itself is capable of acting as an oxidizing agent.

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# BORON AS A FACTOR IN THE CALCIUM METABOLISM OF THE CORN PLANT<sup>1</sup>

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That a relation exists between boron in the substrate and the calcium metabolism of plants has been recognized (3). Practical value, as well as scientific interest, is involved in the subject. Much of the work that has been done is descriptive, dealing with disease symptoms exhibited by the external characters of the plants (6), but some excellent quantitative work also has been accomplished (7, 13). The problem is difficult and requires special methods in its approach. The purpose of this investigation is to study, chemically and structurally, the effect of boron on the calcium metabolism of corn plants.

## PLAN OF EXPERIMENT

Corn seeds of Croshaw's strain of Reed's yellow dent were germinated on moist blotting paper. The seedlings, when large enough to be handled, were transferred to purified sand in highly glazed pots. The sand had been washed successively with 5 per cent HCl, with water, with 5 per cent NaOH, and with distilled water. Tests showed the glazed surfaces of the crocks to be free from traces of any soluble contaminating substances (7). Six series of cultures were established.

During the first week of growth the plants in all series were supplied with a culture solution containing all the required elements except boron, which was omitted from the solution in order that the plants might exhaust any traces available in the seed or present from external sources.

During the second week of growth, the plants in all series were supplied with the complete culture solution, including 0.25 p.p.m. of boron in the form of boric acid. At the end of the second week, all the plants were growing vigorously and appeared to be normal in every respect.

Boron and calcium treatments were started at the beginning of the third week and were continued for 10 days. These treatments are indicated in the description of the solutions presented in table 1. It will be observed that calcium was omitted from some of the solutions and that the boron treatments included a range of concentrations from 0 to 5.0 p.p.m. of this element. In series I and II, calcium nitrate was replaced by sodium nitrate, in equivalent

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

molecular proportions, in order to supply the nitrate ion and to eliminate the calcium ion from the solution. One liter of solution was dripped daily on the sand surface of each culture, and each culture was flushed daily with 500 cc. of fresh solution (9, 11). As a result, the pH of the solutions drained from the cultures varied only from 4.1 to 4.2, as indicated colorimetrically with brom-cresol green.

Microchemical tests of the plant tissues were begun when the plants were 20 days old and were continued daily until the plants were harvested, at the age of 25 days.

TABLE 1  
*Solutions used with six series of corn plants grown in sand culture*

SERIES	MOLAR CONCENTRATIONS OF MAJOR SALTS					TRACE ELEMENTS	
	$\text{KH}_2\text{PO}_4$	$\text{Ca}(\text{NO}_3)_2$	$\text{NaNO}_3$	$\text{MgSO}_4$	$(\text{NH}_4)_2\text{SO}_4$	Boron	Fe, Mn, Zn
I	0.0023	.....	0.0090	0.0023	0.0007	p.p.m. 0.00	p.p.m. 0.25
II	0.0023	.....	0.0090	0.0023	0.0007	0.25	0.25
III	0.0023	0.0045	.....	0.0023	0.0007	0.00	0.25
IV	0.0023	0.0045	.....	0.0023	0.0007	0.10	0.25
V	0.0023	0.0045	.....	0.0023	0.0007	0.25	0.25
VI	0.0023	0.0045	.....	0.0023	0.0007	5.00	0.25

#### CHARACTER OF PLANTS

By the sixth day of treatment, definite symptoms of boron deficiency and toxicity were apparent in some of the series.

At the end of the 10-day treatment, those plants supplied with 0.1 p.p.m. boron and 170 p.p.m. calcium (series IV), as well as those supplied with 0.25 p.p.m. boron and 170 p.p.m. calcium (series V), were large and were devoid of pathological symptoms.

The series of plants supplied with 5.0 p.p.m. boron and 170 p.p.m. calcium (series VI) yielded plants the leaf margins of which, at the end of the 10-day treatment, were brown and dead. The leaves lacked the fresh luster appearing in the normal plants.

In each of the other series—0 p.p.m. boron and 170 p.p.m. calcium (series III), 0 p.p.m. boron and 0 p.p.m. calcium (series I), and 0.25 p.p.m. boron and 0 p.p.m. calcium (series II)—the plants produced symptoms associated with boron or calcium deficiencies. Those plants grown in cultures without boron and without calcium were dying at the time of harvest. The disease symptoms in these plants are small, withered leaves, mottled with red and white blotches, and cracked leaf blades, exuding a red sticky fluid, which in several instances dripped from the lesions. The liquid was abundant and contained dissolved salts. Plants supplied with 0 p.p.m. boron and 170 p.p.m. calcium in the substrate presented white stripes in the leaves, which also showed a green and white mottling, whereas plants supplied with 0.25 p.p.m. boron and 0 p.p.m.

calcium within the substrate presented seared leaf margins and were speckled with white blotches. Little red color was present in the plants of these two series. In general, the red color of the leaves appears to accompany a condition of soluble calcium deficiency within the plant. This would indicate that soluble calcium is available calcium. Boron deficiency is first indicated externally by the presence of white stripes in the leaves and an abnormal condition of the terminal growing point; brown, dead leaf margins are characteristic of the older leaves of the boron-toxic plants.

The plant roots from cultures receiving 0.1 p.p.m. boron and 0.25 p.p.m. boron together with 170 p.p.m. calcium (series IV and V respectively) were long, silvery white, and apparently healthy.

Only a few short, thick roots, deep brown in color, were produced by the plants supplied with 5.0 p.p.m. boron and 170 p.p.m. calcium (series VI) during the experimental period.

Plants of the series deficient in boron but having optimum calcium (series III) produced stubby roots bearing a few short laterals, whereas those deficient in calcium but having optimum boron (series II) yielded, at the time of harvesting, only a few thick brown roots bearing numerous thick fingerlike branches at their terminals. The plants grown in the minus-boron and minus-calcium solution (series I) produced short, thick, brown disintegrating roots.

#### EXPERIMENTAL METHODS USED WITH THE PLANT MATERIAL

Both fresh and formalin-preserved plant materials were used for microscopic work, and some of the material was frozen for the determination of calcium and of boron in the tissues and tissue juices. Oven-dried plants were used for the determination of total calcium and total boron. Total green weights and total dry weights per plant top and milligrams of calcium, of soluble calcium, of boron, and of soluble boron per gram of dry tissue were determined and are recorded in table 2.

The volumetric method of the Association of Official Agricultural Chemists (1, pp. 123-124; 7), slightly modified, was followed for the calcium analyses. The Berger and Truog (2) colorimetric method, also slightly modified, was used for the quantitative determination of boron. This method was modified by diluting the standards and test solutions with sulfuric acid to 50 cc. each, instead of using 10-cc. quantities. These 50-cc. portions were held in clear Nessler tubes and compared over a 20-watt fluorescent daylight lamp.

In order to determine the soluble calcium or boron in the plant juices, 25-gm. samples of fresh tissues were frozen quickly at the time of harvest. The frozen samples were thawed and then were subjected to a pressure of 1250 pounds per square inch for 5 minutes in a Carver press. The juices were immediately filtered, and the entire residue was dried for analysis. The soluble calcium or boron in the juices was determined by subtracting the quantity obtained by analysis of the entire residue of an extracted sample from the total quantity obtained by analysis from an unextracted sample.



Crystal counts of calcium and of boron were made on meristematic growing points, leaves, stems, and roots, of both incinerated and unincinerated formalin-preserved tissues, as well as on fresh tissues. These tissues were prepared as follows: longitudinal sections 10  $\mu$  thick were cut with the freezing microtome while some others were hand-sectioned and treated directly on microscope slides. The sections to be incinerated were placed in the electric muffle furnace at 350°C. for 3 hours.

For calcium crystal counts of the incinerated tissues, the ash was treated with dilute solutions of sulfuric acid or ammonium oxalate. Frequently the calcium compound crystals were stained with 2 per cent alizarin solution in order to bring them into contrast with other crystals that appeared on the slides (4). For the identification of boron in crystal form, the ash on the slide was treated with a dilute solution of hydrochloric acid to set free the boron, followed by a concentrated solution of potassium periodate or potassium iodide; later it was treated with concentrated potassium chloride solution and finally stained with 50 per cent alcoholic alkaline turmeric acid. All slides were air dried before microscopic examinations were made. This is the method described by Chamot and Mason (4), except that fluorine instead of iodine compounds are used as reagents by those authors.

In order to estimate the numbers of boron or of calcium crystals within the tissue cells, similar procedures were followed as with the incinerated tissues. In most cases, however, a cover slip was fastened over the section by means of Canada balsam in order that the highest magnification of the microscope could be employed. The same methods used in testing the plant tissues to identify calcium or boron crystals were applied to chemically pure stock salts of calcium and boron as a means of comparison and check.

Fat in the growing points was located in longitudinal sections by means of both alcoholic and ether preparations of Sudan III stain.<sup>2</sup> Permanent mounts were made of these preparations after dehydration with diethylene oxide. In order to verify the fat reaction, a 1-gm. sample of plant tissue was boiled with dilute potassium hydroxide, evaporated to dryness, and extracted with water, and the filtered extract was acidified with hydrochloric acid. The white precipitate of fatty acid was an indication of the presence of fat in the tissue. This white precipitate was removed, dried, weighed, and calculated in terms of milligrams per gram of dry plant tissue.

Tissue sections of the meristematic growing points were treated for pectin in the same general manner as that used for determining the presence of fat, except that the stain employed to indicate the presence of pectin was ruthenian red.<sup>2</sup> These tissues were dehydrated with diethylene oxide, and a cover slip was sealed over the section with Canada balsam.

In order to exhibit the general cellular structure of the growing points, longitudinal sections, stained with methylene blue, dehydrated, and sealed under a cover slip, were used. All pectic substances stain violet with methylene blue.<sup>2</sup>

<sup>2</sup> Eckerson, S. H. Microchemistry. (Mimeographed outline.)

Spot plate tests of fresh growing meristematic tissues indicated that some of the cells in this region were more acid to appropriate colorimetric indicators than were others (8, 12). In general, however, a higher pH appeared to develop in the tissues of plants possessing the highest soluble calcium content than in the tissues of those having a lower soluble calcium content. The pH of the cells in the meristematic tissue ranges from 4.0 for the plants in the 0 p.p.m. boron treatments to 6.2 for the plants in the 5.0 p.p.m. boron treatments (12).

#### RESULTS OF TREATMENTS INDICATED BY CHEMICAL ANALYSES

The results of quantitative tests for total calcium and for soluble calcium as well as for total boron and for soluble boron are given in table 2. The

TABLE 2

*Results of quantitative chemical analyses of six series of corn plants grown in sand culture*

SERIES	TREATMENT		AVERAGE FRESH WEIGHT OF TOPS PER PLANT	AVERAGE DRY WEIGHT OF TOPS PER PLANT	TOTAL Ca PER GRAM DRY TISSUE	SOLUBLE Ca PER GRAM DRY TISSUE	TOTAL B PER GRAM DRY TISSUE	SOLUBLE B PER GRAM DRY TISSUE
	B	Ca						
	p.p.m.	p.p.m.	gm.	gm.	mgm.	mgm.	mgm.	mgm.
I	0.0	0.0	9.33	1.50	3.0	0.3	0.001	0.0005
II	0.25	0.0	20.33	2.60	3.0	1.0	0.008	0.0069
III	0.0	170.0	29.66	2.85	7.6	2.1	0.002	0.0015
IV	0.1	170.0	52.00	5.40	7.7	2.4	0.005	0.0042
V	0.25	170.0	51.00	5.30	8.0	2.8	0.008	0.0070
VI	5.0	170.0	42.00	4.37	7.7	4.2	0.025	0.0232

average green weight, as well as the average dry weight, per plant top is also given.

It appears from table 2, that for those plants supplied with neither calcium nor boron in the substrate (series I) during the treatment period, the supply of soluble calcium and of soluble boron previously acquired had been virtually depleted at the end of the treatment period and that the plants had ceased to develop shortly after the treatments began. If a supply of fresh boron is not continuously transported from the substrate to the terminal growing points, the plant suffers retardation in growth, characteristic of boron deficiency (5, 10). This observation is supported by the fact that plants suffering from boron deficiency show few boron crystals within the cells of growing tips.

In comparison with the plants of series I, in which no additional boron was supplied to the substrate, the plants of series II, receiving 0.25 p.p.m. boron and 0 p.p.m. calcium during the experimental interval, maintained in a soluble condition a greater proportion of the calcium already present at the beginning of the experimental period. It is evident that the presence of adequate available boron within the plant tends to maintain in an available

condition the calcium previously acquired, even though no fresh supply of calcium is added to the substrate.

The plants of series III, receiving 0 p.p.m. boron and 170 p.p.m. calcium, indicate that nearly one third of all the calcium per gram of dry tissue is in a soluble condition. There is twice as much boron per gram of dry tissue in the plants of series III as in the plants of series I, although the total boron in the plants of these two series is extremely low since no boron was supplied during the treatment period. Much of those small quantities of boron is in a soluble condition. The explanation of the higher quantity of boron in plants of series III, compared with those in series I, is to be sought in the condition of the plants. As was previously pointed out, the plants in series I were characterized by disrupted leaf, stem, and growing point tissues, which suffered loss of tissue fluids through exudation. It was established, by test, that traces of the boron contained in the plant were lost from the tissues by way of the exuded liquids. No such waste of fluids occurred from plants in series III. At the beginning of the treatment period, series III plants contained a sufficient supply of soluble boron and of soluble calcium to promote slight growth throughout the 10-day period of treatment. This growth, however, occurred not at the terminal growing point, but in the older leaves.

It will be observed that the data of fresh weight, dry weight, and total calcium for the plants of series IV are similar to those of series V, and higher than those for any of the other series, in spite of the fact that the boron concentration of the substrate in these two series is only 0.1 p.p.m. and 0.25 p.p.m. respectively. This indicates that the boron requirement of these plants lies within a narrow range of concentrations approximately limited by the concentrations employed in the solutions of these two series. It further indicates that the boron content of the substrate, within certain limits, does not influence significantly the calcium absorption rates of the plants. This is emphasized by the fact that the plants from the four series, III, IV, V, and VI, in which the calcium concentration of the substrate was equal (170 p.p.m.), yielded upon analysis average total calcium values which differed only very slightly. The soluble calcium content of the plants from series IV and V is strongly influenced, however, by their boron content, soluble or total or both, which in turn, is determined by the boron concentration in the substrate.

It must be concluded from the data in table 2, therefore, that soluble calcium is determined not by the total calcium content of the plants, but by the boron content. The data also show that a very large proportion of the boron in the plants is soluble and that the soluble boron is directly related to the total boron in the plants and to the boron content of the substrate in which the plants were grown.

It is evident from table 2 that there is a strong tendency on the part of boron in the plant to maintain in a soluble and, therefore, in an active and available form the calcium that is within the plant. This condition is par-

ticularly exemplified in series IV, V, and VI. In the plants of series VI, the soluble calcium content is approximately twice as high and the boron content is five times as high as the corresponding contents in the normal plants of series IV.

#### MICROCHEMICAL INVESTIGATIONS

An attempt was made by microchemical methods to differentiate between active and nonavailable calcium in the tissues of corn as a check against the analytical methods by which soluble and total calcium were determined. By selecting small pieces of tissue from different parts of the plant it was possible to locate, in the individual plants from the various series, regions of calcium and boron accumulation.

For these investigations whole plants were harvested at the end of the 10-day treatment period and preserved in 10 per cent formalin for future use in permanent mount preparations. Small pieces of living tissue, also selected from various parts of the plant, were sectioned with a freezing microtome, and the sections were treated immediately without formalin preservation. Counts were made of the relative numbers of calcium and boron crystals in these preparations. Cut sections were placed on slides, treated, and incinerated at 500°C. in an electric muffle furnace. After cooling, the ash was treated as previously described, and crystal counts, representing the total calcium or boron of the tissue section, were made. Slide preparations were frequently made, on succeeding days, of root, stem, and leaf tissues selected from the plants of the different series.

Invariably all slide sections of corresponding tissue from plants receiving the same treatment yielded calcium crystal counts which were fairly uniform in numbers. Root tissues always yielded the highest numbers of crystals; stem tissues, the lowest; and leaf tissues, numbers intermediate between these two.

Slide preparations of incinerated tissue sections from plants treated with 5.0 p.p.m. boron in the substrate yielded the highest boron crystal counts, those from plants of the cultures without boron in the substrate yielded the lowest crystal counts, and those from plants taken from cultures treated with the optimum range of boron concentrations yielded crystal counts corresponding in number to the boron concentration in the substrate. These results indicate that the total boron in the ash of the tissue sections is directly correlated with the corresponding boron concentrations in the substrate. This is in perfect agreement with the quantitative chemical analyses of the tissues.

The examination of all slides, prepared for estimating calcium in tissue sections by the method of incineration, uniformly showed calcium crystal counts approximately equal in numerical value, for corresponding tissue sections taken from plants of series III, IV, V, and VI, in which the calcium concentration of the substrate was 170 p.p.m. The crystal count method,

therefore, shows no relation between boron accumulation and total calcium in the tissues.

Since the method of incineration dealt only with "total" quantities and did not differentiate between soluble or functional calcium and insoluble calcium, sections of living tissues were treated and prepared for crystal counts in the cells as described.

Groups of ten cells, in prepared and treated leaf tissue sections taken from plants grown with deficient boron (0.0 p.p.m.), optimum boron (0.1-0.25 p.p.m.), and excess boron (5.0 p.p.m.) treatments, were selected, and calcium crystal counts were made. Tissues from boron-deficient plants yielded an average of 16.9 crystals per cell; those from cultures in the optimum range of boron treatment, 39.5 crystals; and those from the excess boron treatment, 67.8 crystals. The calcium crystals were rather uniformly distributed throughout the cytoplasm. This ascending scale of crystal counts was always much lower in numerical value and not in agreement with the very uniform calcium crystal arrangement that appeared on the slides of the incinerated leaf tissue taken from plants treated with the corresponding boron concentrations. It is evident that the ascending scale of crystal counts does not here represent total calcium present in the individual cells, but may be regarded as representing the soluble calcium fraction of the cells.

Counts were next made of the boron crystals that were formed in cells of the fresh leaf tissue taken from the same plants tested for calcium crystal counts. Numerous preparations for boron crystal counts of both stem and leaf tissues, as for calcium crystal counts, were made on different days. Tissues of plants from the minus-boron treatments yielded few or no boron crystals in the cells; those from the optimum range of boron treatments and those from excess boron treatments yielded crystal counts which were roughly proportional to the boron concentrations in the substrate. From these tests it appears that the concentration of boron in the growth substrate determines its accumulation in the plant. This was indicated by the crystal counts of both incinerated tissue and fresh tissue. This again agrees well with the data of quantitative chemical analyses.

These relations are of special interest when considered in connection with the fact that the plant is unable to store a surplus of available boron within its tissues and subsequently to make effective use of it. In this connection it is to be noted that the plants grow normally only within the optimum range of boron concentrations in the substrate and within the corresponding effective range of concentrations within the plants. Below this range, plants show pathological symptoms of deficiency; and above this range, they exhibit the characteristic symptoms of toxicity.

The boron crystal counts present a picture quite different from that of the calcium crystal counts. On the slides of incinerated tissue sections calcium crystals showed no relation whatever with the boron crystal counts. This was in perfect agreement with the quantitative chemical analyses of the tissues

for total calcium. The results of these analyses, it will be recalled, show fairly uniform values for corresponding tissues of all plants throughout the series except those for tissues of plants grown in cultures deficient in calcium (series I and II). When, however, the calcium counts were made in the cells of tissue sections not subjected to incineration, the average calcium crystal count per cell followed the order of count magnitude that was obtained for the corresponding boron crystal counts. Low, medium, and high calcium crystal counts corresponded respectively with low, medium, and high boron crystal counts. This shows a very definite relation between boron content of the tissue cells and active or functioning calcium, assuming that counts of calcium crystals within the cells of tissue sections represent active calcium.

During the course of microchemical investigations of the terminal meristem tissues of the plants it was discovered, incidentally, through the use of the staining methods here employed that pronounced differences in pectin content and in fat content occurred in these active tissues of the plants from the different series. An attempt was made, therefore, to determine qualitatively, by the staining methods already described, whether the differences in the pectin and the fat content of the cells of these terminal regions might be related to the boron treatments which the plants received. The stains employed in these tests, ruthenian red and Sudan III, are considered to be general stains for pectin and fat respectively. Because both of the organic compounds, pectins and fats, may be present within the plant tissues in various forms, the interpretation of results of these tests must be considered to be of a preliminary nature, and they will require confirmation by the use of much more exact methods of analyses. It is interesting, however, that the meristematic tissues of plants supplied with 0 p.p.m. boron and 170 p.p.m. calcium (series III) always yielded, throughout the cellular cytoplasm, positive tests for pectins and negative tests for fat, whereas those plants supplied with 5.0 p.p.m. boron and 170 p.p.m. calcium (series VI) always yielded negative tests for pectin within the cellular cytoplasm and positive tests for fat. The presence of both fat and pectin was indicated in the meristematic tissues of those plants grown in series IV and V, in which the boron concentration of the substrate was 0.1 p.p.m. and 0.25 p.p.m. respectively and the calcium content 170 p.p.m.

Preliminary qualitative tests are strongly suggestive of an important relationship between boron of active corn tissues and their pectin content, and between boron and fat metabolism in which calcium is involved.

In this preliminary investigation with a representative monocot (corn) much consideration was given to the selection and development of the most appropriate methods to be followed in the analyses for boron and calcium in the plant tissues, and much time and energy were given to the thorough testing of the suitability of the methods selected. These investigations are being continued by means of a comparative study of representative monocots and dicots.

## SUMMARY

The optimum boron requirement of corn plants grown in sand supplied with culture solution by the continuous flow method lies within a narrow range of concentrations approximately limited by the concentrations employed in the solutions containing 0.1 p.p.m. to 0.25 p.p.m.

The presence of adequate available boron within the corn plant tends to maintain the calcium, previously acquired, in an available condition even though no fresh supply of calcium is provided in the substrate.

The boron content of the substrate, within certain limits, does not influence significantly the calcium absorption rates of the plants.

The soluble calcium in corn tissues is determined not by the total calcium of the plants, but by the boron content, which, in turn, is determined by the boron content of the substrate.

A very large proportion of the boron in the plants is in a soluble form. This proportion of soluble boron is directly related to the total boron in the plants and to the boron content of the substrate.

The plants grow normally only within the optimum range of boron concentrations in the substrate and within the corresponding effective range of concentrations within the plants. Below this range, plants show pathological symptoms of deficiency, and above this range they exhibit the characteristic symptoms of toxicity.

Repeated observations made on the plants indicated that a high fat content together with a low pectin content of the terminal meristem tissues accompanied a condition resembling early maturity of the plant. It is thus apparent that these preliminary tests are strongly suggestive of an important relationship between boron of these active tissues and their pectin content and between boron and fat metabolism.

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# THE PHOSPHORUS CONTENT OF A SANDY LOAM CONTAINING SUFFICIENT AVAILABLE PHOSPHORUS FOR VEGETABLE CROPS

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Fertilizer applied to vegetable crops usually contains very much larger amounts of phosphorus than the crops remove. An application of 1,000 to 2,000 pounds per acre of a mixture containing 8 per cent of phosphoric acid supplies 80 to 160 pounds; crops remove only 10 to 30 pounds per acre. The annual residue is, thus, 50 to 150 pounds.

As is well known, much of this residue is fixed in the plowed zone. For example, Hester (4) reported that truck soils in the Norfolk, Virginia, district contained in the top 7 inches about 4,000 pounds per acre more phosphoric acid than that found in virgin soil; the second 7 inches contained about 1,300 pounds more than the same horizon of virgin soil (table 1).

With accumulations of this magnitude it might be anticipated that the phosphorus-fixing capacity of the soil would, sooner or later, become exceeded and that the application of excess phosphorus would cease to be necessary.

In the literature are found occasional reports of fertilizer tests in eastern states where there was no response to phosphate in the fertilizer. Martin, Brown, and Sprague (5) conducted an experiment with potatoes in New Jersey in which there was little or no increase in yield from phosphate in the fertilizer. Similarly, Brown (2) in Connecticut obtained no response with potatoes from phosphate applications on an old tobacco soil. In Rhode Island, Odland and Crandall (6) applied 1,500 pounds per acre of 3-12-4 to cabbage for 10 years, then changed to an 8-0-8. For the following 8 years, the 8-0-8 averaged 482 barrels of cabbage per acre while an 8-8-8 produced at the rate of 474 barrels. None of these workers reported either the total or available phosphorus of their soil. Working from a soil viewpoint, Hester (4) estimated the phosphorus-fixing capacity of Sassafras sandy loam at the Norfolk Truck Experiment Station to be about 5,000 pounds of  $P_2O_5$  per 2,000,000 pounds of soil and found an average of 4,484 pounds present in growers' fields (table 1). One field showed over 6,000 pounds. This field was considerably above its probable phosphorus-fixing capacity.

## RESULTS ON CHENANGO FINE SANDY LOAM IN OHIO

Similar data have been obtained at the Washington County Truck Experiment Farm near Marietta, Ohio, on Chenango fine sandy loam, a terrace soil

of the Muskingum River. A fertilizer experiment with four vegetable crops was started in 1915 on a field said to be in a low state of fertility as compared with neighboring truck farms. The details of the experiment have been published (3).

At the outset it was found that liming alone made phosphorus amply available for sweet corn, but the other three crops, tomatoes, cabbage, and cucumbers, gave a distinct response to phosphate fertilizer. These three crops also gave increases in yield to superphosphate supplementing 16 tons of manure per acre, but the increases were small and continued for only a few years. During the second 8 years of the experiment, the manure alone supplied ample phosphorus for all the crops (table 2); in other words, with manure supplying approximately 64 pounds of phosphoric acid a year, the accumulated residue reached the point where there was sufficient available, with continued manuring, for all the crops. Incidentally, all these plots were limed at the rate of a ton of

TABLE 1  
*Total phosphoric acid in soils of the Norfolk Trucking District*  
Data of Hester (4)

HORIZON	APPROXIMATE DEPTH  <i>inches</i>	PARTS $P_2O_5$ PER 2,000,000 OF SOIL	
		Cropped soils	Virgin soils
A <sub>1</sub>	2-7	4,484	590
A <sub>2</sub>	7-14	1,714	396
B	14-21	772	534

ground limestone annually. The pH was about 7.0 at the end of the eighth year. This was, of course, a factor in the peculiar availability of the phosphorus.

At the end of 16 years some of the fertilizer treatments were changed. One unmanured plot that had annually received 640 pounds per acre of 4-10-4 was changed to 1,000 pounds of 8-0-8. Surprisingly, for the following 4 years all the crops gave good yields on this plot with no indications of phosphorus deficiency. The yields of tomatoes and cucumbers began to decline in the fifth year, but the yields of cabbage as well as sweet corn were maintained into the seventh and eighth years (table 3). Thus an annual application during the first 16 years of 400 pounds per acre of 16 per cent superphosphate resulted in a considerable residue of highly available phosphorus.

#### *Total and available phosphorus in the soil*

Soil samples were collected at the end of the sixteenth year when the changes were made in the fertilizer plan. Total phosphorus was determined by Zinzadze's procedure (8); readily available phosphorus by Truog's method (7); and, in addition, water-soluble phosphorus was determined by shaking 1 gm.

of dry soil in 200 cc. of water for 30 minutes, then proceeding as in Truog's method. Data from the plots of special interest are given in table 4.

TABLE 2

*Effect on yield from applying superphosphate with manure*

All yields calculated to pounds of marketable produce per acre

CROP AND TREATMENT*	AVERAGE ANNUAL YIELD	
	First 8 years 1915-1922	Second 8 years 1923-1930
Cabbage, manure only.....	20,005	24,263
With superphosphate.....	20,907	24,360
Increase.....	902	97
Tomatoes, manure only.....	13,803	12,895
With superphosphate.....	14,485	13,180
Increase.....	682	285
Cucumbers, manure only.....	18,576	21,754
With superphosphate.....	19,340	21,623
Increase.....	764	-131
Sweet corn, manure only.....	7,960	9,263
With superphosphate.....	7,960	9,023
Increase.....	0	-240

\* All plots annually manured at rate of 16 tons per acre. Annual applications of 16 per cent superphosphate at the rate of 400 pounds per acre.

TABLE 3

*Yields after phosphate was omitted from fertilizer*

Pounds of marketable produce per acre

	AVERAGE ANNUAL YIELD FOR 4 YEARS (1931-1934)		AVERAGE ANNUAL YIELD OF SEVENTH AND EIGHTH YEARS, 1937-1938	
	Continuously phosphated— plot 34	No phosphate after 1930— plot 31	Continuously phosphated— plot 34	No phosphate after 1930— plot 31
Tomatoes.....	7,915	8,176	11,900	10,710
Cucumbers.....	3,110	3,900	15,650	11,630
Cabbage.....	22,920	24,420	31,920	33,000
Sweet corn.....	4,350	4,600	8,840	8,460

In view of the large amount of available phosphorus shown by these analyses, and particularly the large amount in the water extracts, it is not surprising that crops on plot 31 had ample phosphorus for several years after phosphate was omitted from the fertilizer.

Growers' soils in the Marietta district appear to be similarly high in avail-

able phosphorus. Six fields were sampled in the fall of 1938; three were said to have been heavily fertilized, three lightly fertilized. The water-extractable phosphorus in these samples ranged from 50 to 200 pounds per acre.<sup>1</sup>

TABLE 4  
*Phosphorus content of the soil after 16 years of fertilizer treatments*

PLOT NUMBER	ANNUAL ACRE APPLICATION	PHOSPHORUS PER 2,000,000 POUNDS OF SOIL		
		Total	Available	Water-soluble
		<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
29	None	1,260	100	40
31	640 pounds 4-10-4	1,460	200	100
34	640 pounds 4-10-0	1,560	200	120
28	32,000 pounds manure	1,400	220	90

TABLE 5  
*Phosphorus content of the surface and the subsurface soil in the twenty-fourth year of the experiment*

Pounds of phosphorus per 2,000,000 pounds of soil

PLOT NUMBER	TOTAL PHOSPHORUS APPLIED IN 24 YEARS*	WATER-SOLUBLE PHOSPHORUS IN SURFACE SOIL	TOTAL PHOSPHORUS	
			In surface soil, 0 to 6 inches	In subsurface soil, 9 to 15 inches
33	0	40	1,200	780
32	419	60	1,380	740
31	447	50	1,390	840
34	1,144	100	2,105	930
28	1,325†	100	1,830	830

\* Calculated from the "guaranteed analysis" of available phosphoric acid of the superphosphate. The actual total phosphorus was probably slightly higher.

† Based on an estimate of 4 pounds of  $P_2O_5$  per ton of manure, apparently too high an estimate.

#### *Retention of phosphorus in the plowed soil*

With such large amounts of phosphorus readily soluble in water, it seemed that there might be some leaching and that the leached phosphorus would be

<sup>1</sup> To check on the writer's technic and particularly to discover any error that might be due to the presence of elements other than phosphorus, R. H. Simon, of the soils department of this institution, also made water extracts of these six samples. He soaked 5 gm. of soil in 500 cc. of water for 24 hours, instead of merely shaking for 30 minutes, then determined the extracted phosphorus by Zinzadze's method. In all samples he found somewhat larger amounts than those obtained by the writer.

The empirical procedures used here were adopted merely to demonstrate the unusually large amounts of water-extractable phosphorus in these soils. They were not, of course, strictly quantitative estimates. Repeated extractions gave larger amounts. For example, with two samples, Simon obtained the following data (in parts per 2,000,000):

found fixed in the subsurface. Hester's data as given in table 1 indicate some such movement. The first subsurface samples were collected in the summer of 1938, the twenty-fourth year of the experiment.<sup>2</sup> The surface soil samples included the top 6 inches; the subsurface, a measured depth of 9 to 15 inches. Plow depth was about 8 inches.

The data (table 5) showed surprisingly little movement of phosphorus into the subsurface even in the continuously phosphated plots with 100 pounds of water-soluble phosphorus per 2,000,000 of surface soil. Moreover, if the crops removed 5 to 10 pounds of phosphorus annually, the remainder was accounted for chiefly by the accumulation found in the plowed horizon.

The interesting question as to how the phosphorus can be partly soluble and yet be retained has not been studied. The point of chief interest here is that when this soil was phosphated to a point beyond the requirements of the crops a large part of the excess remained available in the surface soil instead of leaching downward.

#### DISCUSSION AND CONCLUSIONS

The relatively rapid accumulation of available phosphorus in Chenango fine sandy loam might be attributed either to an initially high phosphorus content or to a low fixing capacity. The phosphorus content of the unfertilized plots is about 1,300 pounds per 2,000,000, which is not high compared with some Ohio soils. Of 160 soil samples from diverse points in Ohio analyzed by Ames and Gaither (1), 43 were found to contain over 1,300 pounds per 2,000,000. A relatively low phosphorus-fixing capacity is, therefore, characteristic of this soil. When the phosphorus content was raised to only 1,500 pounds per 2,000,000, the available supply was sufficient for the crops grown in this experiment.

The amount of phosphate fertilizer needed to maintain yields once the phosphorus level has been raised to this point has not been definitely established by field tests. Presumably it would not be necessary to apply more than the crops remove. Such applications would be a marked departure from current fertilizer practice. Limited data from applications of 8 tons of manure per acre, supplying perhaps 10 to 15 pounds of phosphorus, indicate, however, that this was not quite sufficient to maintain maximum yields of tomatoes and cabbage; but 16 tons of manure supplied more than needed, as indicated by the data of table 2.

The tentative recommendation is to include about 200 pounds per acre of

	<i>Sample A</i>	<i>Sample B</i>
First extraction.....	90	125
Second extraction.....	50	86
Third extraction.....	24	31
Total of three extractions.....	164	242

<sup>2</sup> Most of the analyses were made in the laboratory of G. M. McClure, of the soils department of the Ohio State University.

20 per cent superphosphate, or its equivalent, in the fertilizer for unmanured soil.

Incidentally, the data show the differences in the phosphorus level required by the different crops grown here. Sweet corn thrived on soil with as little as 20 pounds of water-soluble phosphorus (determined by the procedure described); cabbage required about 50 pounds; cucumbers about 70 pounds; and tomatoes needed about 100 pounds per 2,000,000 pounds of soil.

#### SUMMARY

On Chenango fine sandy loam in southeastern Ohio an annual application of 400 pounds per acre of 16 per cent superphosphate for 16 years resulted in an accumulation of available phosphorus sufficient to maintain the yield of tomatoes and cucumbers for 4 years and the yield of cabbage and sweet corn for 8 years.

When the phosphate applications were discontinued, the total phosphorus content of the soil was about 1,500 pounds per 2,000,000, of which 100 to 120 pounds were readily extracted by water.

There was little or no leaching of phosphorus into the soil at a depth of 9 to 15 inches. Most of the applied phosphorus was accounted for by the estimated removal in the crops and by the accumulation in the plowed horizon.

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# ORGANIC PHOSPHORUS IN SOILS: I. THE EXTRACTION AND SEPARATION OF ORGANIC PHOSPHORUS COMPOUNDS FROM SOIL<sup>1</sup>

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## GENERAL INTRODUCTION

The presence in soil of phosphorus in organic combination has been established beyond doubt. In the surface layers of many soils, 30 to 85 per cent of the phosphorus is organic (2, 19, 24, 40). The nature of the compounds which so exist has not been fully established, however, and there is confusion with regard to the behavior and the significance of these constituents.

Many of the soils in which the concentration of organic phosphorus is high are very deficient in available phosphorus. It is therefore clear that growing plants do not utilize directly to any appreciable extent the organic phosphorus compounds of soil. Plants take up phosphorus chiefly as the monophosphate ion,  $\text{H}_2\text{PO}_4^-$  (20, 25, 26, 36), and the dependence of crops on an adequate supply of readily soluble phosphate is now generally recognized. Hence it appears likely that, in order to be utilized, organic phosphorus must first be mineralized, and that the availability of organic phosphorus compounds in the soil is directly related to their decomposition.

When organic matter is incorporated in soil a rapid decomposition ensues, in the earlier stages of which fungi play an important part (5, 34, 35, p. 596). In the presence of soluble phosphate a considerable synthesis of organic phosphorus takes place simultaneously (5, 33). After the utilization of most of the easily decomposed energy material, such as hemicelluloses and cellulose, bacterial decomposition becomes dominant, and under these conditions a part of the organic phosphorus may be mineralized again. Thus organic phosphorus in the soil may originate from the organic residues added to soil, and also through synthesis by microorganisms.

Little information is available as to whether the relatively large quantity of organic phosphorus in soil is mainly an accumulation of inactive and resistant end-products, or whether as a whole it plays an active part in a phosphorus

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cycle, being continuously synthesized by microorganisms and replenished from plant residues, and simultaneously being decomposed. Some light may be thrown on this situation by a preliminary consideration of the phosphorus compounds likely to occur from time to time in the soil.

Five classes of organic phosphorus compounds, originating from plant residues and microorganisms, could conceivably occur in soils; phospholipids, sugar phosphates and related compounds, phosphoproteins, nucleic acids, and phytin and its derivatives. The evidence available indicates that, under suitable culture conditions, all these types of compounds are readily attacked by enzymes which are present in soil microorganisms (7, 8, 15, 17, 21, 27). Similarly, the phosphorus of these compounds is assimilated by plants from culture solutions, as shown by Schreiner and Skinner (25) and by Weissflog and Mengdehl (36), although the latter workers found that phytin phosphorus was the least readily utilized. Auten (3) showed that lecithin, nucleic acid, and phytin were decomposed to the extent of 60 to 80 per cent in sand cultures, and Heck and Whiting (12, 37) found that phytin phosphorus was utilized by oats and clover in sand cultures.

Consideration of these findings makes it very difficult to assume an accumulation of stable forms of organic phosphorus in the soil. On the other hand, it seems equally difficult to suppose that high concentrations of labile compounds can be maintained through synthetic activities. Perhaps it is significant that the foregoing conclusions are mainly qualitative and are based on observation of artificial cultures, where conditions peculiar to the soil could not exert their influence.

Very little is to be found in the literature relating to the fate of organic phosphorus compounds in the soil itself. Hilbert et al. (13) and Spencer and Stewart (31) have recorded results which indicate that glycerophosphates are readily mineralized in the soil. Kelley (16) and Neubauer (18) observed that casein was rapidly decomposed in soil. According to Neubauer, the phosphorus of nucleic acid was about 70 per cent assimilated by rye seedlings growing in sand or mixed soil-and-sand cultures. On the other hand, phytin phosphorus was not taken up from soil-and-sand cultures, although it was well utilized in the pure sand cultures.

This last observation suggests that some constituent of the soil influences the utilization of phytin, probably by inhibiting its decomposition. It is well known that phytin forms a highly insoluble ferric salt, a fact which might account for its being "fixed" on coming in contact with soil.

The recent observation of Gulland and Jackson (11) that ribonucleic acid is only 75 per cent dephosphorylated by various combinations of enzymes, leaving a residue of unknown constitution, appears to be of the greatest significance in this context. Should this be true of the nucleic acid decomposition taking place in soil, it would account for the occurrence in soil of stable and inert nucleic acid derivatives.

Some work has been done directly on the separation and identification of the

organic phosphorus compounds in soil. Ether-soluble phosphorus is known to be present only to the extent of about 1 per cent of the total organic phosphorus (1, 32, 39). Shorey (28) separated material which was considered to be nucleic acid. Bottomley (4) obtained a preparation from peat which he thought was an adenine-uracil dinucleotide. Wrenshall and McKibbin (39) also separated material from which adenine and uracil were obtained. Stoklasa (32) attempted to isolate phytin from an organic soil but failed to obtain sufficient product for purposes of identification.

The investigations which are reported in this series of papers were further efforts to characterize the organic phosphorus compounds of soil. The problem has been approached in two ways, by direct attempts to separate and identify the soil products, and by consideration of the decomposition of organic phosphorus compounds in the soil itself. Throughout the work quantitative considerations have been kept in mind.

#### ANALYTICAL METHODS

The methods of phosphorus determination employed were developed in this laboratory specifically for the purpose of conducting these investigations. The photoelectric technic for inorganic phosphate colorimetry developed by Dyer and Wrenshall (9) and further elaborated by Smith et al. (30) makes possible accurate determinations even in highly colored soil extracts. Organic phosphorus in soils and soil extracts has been determined by the method of Wrenshall and Dyer (40) in which organic phosphorus represents the difference between inorganic phosphate determined directly and total phosphorus determined after  $\text{Mg}(\text{NO}_3)_2$  fusion.

#### I. THE EXTRACTION AND SEPARATION OF ORGANIC PHOSPHORUS COMPOUNDS FROM SOIL

When the task of preparing specimens of soil nucleotide material was undertaken, the yields obtained were very disappointing, and it soon became evident that the procedure of Wrenshall and McKibbin was very inefficient when generally applied. Briefly, this procedure consists of the following steps: The soil is leached with *N* HCl to remove calcium and then is extracted with cold 4 per cent  $\text{NH}_4\text{OH}$  for 24 hours. Excess ammonia is evaporated from the extract, and  $\alpha$ -humus is precipitated by acidification with HCl or  $\text{HNO}_3$ . The filtrate is evaporated under vacuum to a small volume, and the nucleotides are precipitated by addition of HCl and a large excess of alcohol. The products so obtained in several experiments contained only 5-10 per cent of the total organic phosphorus in the original soils. Reprecipitation of the  $\alpha$ -humus gave only a further 3 per cent yield. Not only was it difficult to obtain sufficient amounts of material for the work, but it was felt that the ultimate aim of the investigation would not be attained unless a large proportion of the total organic phosphorus could be separated and identified.

Analyses of extracts showed that only about 25 per cent of the organic

phosphorus had been brought into solution by extraction with cold  $\text{NH}_4\text{OH}$ , and furthermore that of this, not more than 60 per cent passed into the filtrate from the  $\alpha$ -humus. These results led to a study of various methods of extraction and of the partition of organic phosphorus between the  $\alpha$ -humus precipitate and filtrate.

#### EXPERIMENTAL

##### *Efficiency of extraction*

A 4 per cent  $\text{NH}_4\text{OH}$  solution and a 1 per cent  $\text{Na}_2\text{CO}_3$  solution were compared as extractants for organic phosphorus, with and without preliminary leaching of the soil with  $N$  HCl. The soil used was Ste. Clothilde muck, 20-gm. samples of which were extracted with 200 cc. of alkaline solution, by shaking occasionally for several hours and finally allowing to stand overnight. For the hot ammonia extraction, the bottles were closed and heated for several hours on a boiling-water bath, with occasional shaking. The mixtures were filtered on Büchner funnels and washed with water. Analyses of the extracts are given in table 1.

From table 1 it may be seen that cold solutions of  $\text{Na}_2\text{CO}_3$  and  $\text{NH}_4\text{OH}$  applied to the unleached soil were very inefficient extractants for organic phosphorus, whereas hot ammonia gave 58.4 per cent extraction. Preleaching with HCl was very effective in increasing the organic phosphorus extracted by the alkaline solution. Extraction with cold ammonia, preceded by acid leaching, was much more efficient in these small-scale experiments than it was in the attempts at isolation. Throughout this work it has been observed that the efficiency of small-scale experiments could not easily be attained when the procedures were applied to larger amounts of soil. Hot ammonia following leaching with HCl completely extracted the organic phosphorus from this soil.

The effectiveness of acid leaching in promoting the alkali solubility of soil organic matter has been known for a long time and has been attributed to the insolubility or resistance to dispersion of calcium and other metal complexes of the colloidal organic material. As regards the organic phosphorus compounds, in the light of our final isolation of phytin (10), the effect may be ascribed in part to the precipitation of calcium phytate in alkaline solution. This supposition is supported by data in table 1. When  $\text{Ca}(\text{NO}_3)_2$  was added to the  $\text{NH}_4\text{OH}$  extract before filtration, the organic matter in the filtrate was lower by 22 per cent, whereas the organic phosphorus was 67 per cent lower, indicating a specific precipitation of organic phosphorus.

The ratios of organic phosphorus to organic matter in the extracts, as given in the last column of table 1, are remarkably uniform with the exception of treatment 8. Hobson and Page (14) found a similar relation between the carbon and nitrogen extracted. Our results are in support of their conclusion that organic matter is bound in the soil in such a way that even the water-soluble constituents may not be brought into solution unless the colloidal

complex as a whole is acted on by dispersing agents. Even after the organic matter is peptized, it is difficult to effect any clear-cut fractionation of the carbon, nitrogen, and phosphorus compounds.

TABLE 1  
*Phosphorus and organic matter in alkaline extracts of muck soil*  
Weights per 100 gm. of soil

TREATMENT	DIRECT P	TOTAL P	ORGANIC P	ORGANIC P AS PER CENT TOTAL SOIL ORGANIC P*	TOTAL SOLIDS	ASH	ORGANIC MATTER	ORGANIC P ORGANIC MATTER
	mgm.	mgm.	mgm.		gm.	gm.	gm.	
DIRECT:								
1. $\text{Na}_2\text{CO}_3$ .....	3.32	3.45	0.13	0.24	5.53	5.16	....	....
2. $\text{NH}_4\text{OH}$ .....	2.29	10.00	7.71	14.0	6.95	1.18	5.77	1.34
Washings.....	2.83	6.85	4.03	7.33	3.32	0.59	2.73	1.48
Total.....			11.74	21.3				
3. $\text{NH}_4\text{OH}$ (hot).....	5.60	37.65	32.05	58.4	22.89	1.84	21.04	1.52
PRELEACHED:								
4. $\text{HCl}$ , $\text{Na}_2\text{CO}_3$ .....	5.66	14.84	9.18	16.7	7.62	4.65	....	....
5. $\text{HCl}$ , $\text{NH}_4\text{OH}$ .....	4.00	31.88	27.9	50.7	18.67	1.17	17.50	1.59
5(a). Second $\text{NH}_4\text{OH}$ ex- traction.....	1.04	11.80	10.8	19.7				
Total.....			38.7	70.4				
6. N/10 $\text{HCl}$ , $\text{NH}_4\text{OH}$ .....	6.00	26.40	20.40	37.1	15.63	2.00	13.63	1.50
7. $\text{HCl}$ , $\text{NH}_4\text{OH}$ (hot).....	5.00	62.00	57.00	103.1	40.66	1.06	39.26	1.45
8. $\text{HCl}$ , $\text{NH}_4\text{OH}$ (hot) + $\text{Ca}(\text{NO}_3)_2$ .....	1.05	21.10	20.10	36.4	35.40	4.71	30.69	0.65

\* Total soil organic P = 55.0 mgm. P per 100 gm. soil.

*Partition of organic phosphorus between humic acid precipitate and filtrate*

The extraction with hot ammonia, preceded by leaching with  $\text{HCl}$ , was tested on several soils, in a further series of small-scale experiments. At the same time the partition of organic phosphorus between the  $\alpha$ -humus precipitate and filtrate was studied. As before, 20-gm. samples of soil were leached with N  $\text{HCl}$ , washed, then digested on the steam bath with 200 cc. of 4 per cent  $\text{NH}_4\text{OH}$ , filtered, and washed. Aliquots of the acid and alkaline extracts were analyzed for organic phosphorus. The alkaline extracts were then acidified

with  $\text{HNO}_3$  to precipitate  $\alpha$ -humus, and the filtrates therefrom were also analyzed for organic phosphorus. The results are given in table 2.

The extraction of organic phosphorus by leaching with  $N$  HCl was of minor proportions. The organic phosphorus obtained in the hot ammonia extracts ranged from 77-92 per cent of the total. The proportion which passed into the filtrate was very small, however, being only from 7.8-10.5 per cent for all except the muck soil, where 28 per cent remained in solution. These results disclosed the second reason why such poor yields have been obtained in our

TABLE 2

*Organic phosphorus distribution in acid and ammonia extracts and in the  $\alpha$ -humus filtrates of various soils*

Weights per 100 gm. of soil

SOIL	ACID EXTRACT				AMMONIA EXTRACT				$\alpha$ -HUMUS FILTRATE			
	Inorganic P	Total P	Organic P	Organic P as per cent total organic P	Inorganic P	Total P	Organic P	Organic P as per cent total organic P	Inorganic P	Total P	Organic P	Organic P as per cent organic P in $\text{NH}_4$ extract
	mgm.	mgm.	mgm.		mgm.	mgm.	mgm.		mgm.	mgm.	mgm.	
Ste. Clothilde												
Muck.....1	5.51	6.99	1.48	2.7	2.70	44.8	42.1	77.0	3.68	15.5	11.8	28.0
Podzol.....2	1.52	3.71	2.19	5.6	5.86	40.8	34.9	90.5	1.38	4.98	3.6	10.3
Macdonald College.....3	37.9	38.8	0.9	2.1	25.6	59.0	33.4	78.0	18.4	21.2	2.8	8.4
Podzol.....4*	1.74	3.65	1.91	...	6.86	52.0	45.1	...	3.20	6.91	3.7	8.2
Podzol.....5*	3.54	5.41	1.87	...	8.38	47.4	39.0	...	4.65	8.04	3.4	8.7
Halliday Podzol, A <sub>1</sub> Layer...6	17.9	21.0	3.1	2.3	9.62	134.0	124.4	91.0	7.83	20.4	12.6	10.1
Halliday (moist).....7	16.1	18.8	2.7	2.4	7.98	109.0	101.0	92.0	6.88	17.5	10.6	10.5
Podzol.....8*	1.90	4.17	2.27	...	8.10	46.8	38.7	...	2.34	5.37	3.0	7.8

\* The organic phosphorus content of soils 4, 5, and 8 was not determined.

attempts at isolation. They were disconcerting because earlier reports (23, 28) indicated that by far the larger part of the organic phosphorus passed into the filtrate, and the data of Yoshida (41), published since this work was completed, show that almost all the organic phosphorus in NaOH extracts of Hawaiian soils escaped precipitation. The distribution may be expected to vary with different types of soil, but there is a suggestion here that it may be more favorable in NaOH than in  $\text{NH}_4\text{OH}$  extracts and may be affected by apparently minor differences in procedure. In this laboratory the use of NaOH as an extractant was abandoned (4, 6, 9, 38) because of the high salt concentrations which finally resulted.

A search was made for some means of obtaining a more favorable distribution of the organic phosphorus in the ammonia extract. The results of Schollenberger (23) indicate that acetic acid causes less precipitation of organic phosphorus than do the strong mineral acids. This was found to be true, but the ratio of organic phosphorus to organic matter in the acetic acid filtrate was about the same as in the  $\text{HNO}_3$  filtrate. This was due to incomplete precipitation of the  $\alpha$ -humus fraction, which then, in the final separation with alcohol, contaminated the precipitate containing the organic phosphorus. The use of acetic acid thus results finally in a disadvantage rather than an advantage.

It was noted that the partition was poorest in the extracts from mineral soils, which suggested that inorganic constituents might be causing the organic phosphorus to precipitate along with the humic acid. A test was therefore made in which ammonium oxalate was added to the alkaline extracts prior to acidification. Ammonia extracts of soils 1, 2, and 7 (see table 2) were prepared as before. To 100 cc. of each extract, equivalent to 3.3 gm. of soil, 0.5

TABLE 3  
*Organic phosphorus in  $\alpha$ -humus filtrates in the presence of oxalate*

Weights per 100 gm. of soil					
SOIL	TOTAL P	INORGANIC P	ORGANIC P	ORGANIC P AS PER CENT OF ORGANIC P OF $\text{NH}_3$ EXTRACT	INCREASE IN RECOVERY OVER $\text{HNO}_3$ ALONE*
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>		
1	34.3	12.7	21.6	36.6	8.6 (31%)
2	24.5	13.1	11.4	28.4	18.1 (175%)
7	70.1	15.3	54.8	52.0	41.5 (395%)

\* Cf. table 2.

gm. of ammonium oxalate was added. The  $\alpha$ -humus was precipitated with  $\text{HNO}_3$ . Analyses of the filtrates are given in table 3.

The effect of ammonium oxalate was to increase the quantity of organic phosphorus passing into the filtrate, while at the same time the separation of  $\alpha$ -humus was as complete as when  $\text{HNO}_3$  was used alone. The difference was of material proportions with the podzol soils, and especially with soil 7, where the proportion of the extracted phosphorus appearing in the filtrate increased to 52 per cent. On reprecipitation of the  $\alpha$ -humus from soil 7 in this experiment only an additional 1.4 per cent of the organic phosphorus was obtained in the filtrate.

The optimum concentration of oxalate was next determined. Varying amounts of oxalate were added to 100-cc. portions, equivalent to 2 gm. of soil, of an ammonia extract of soil 7, and the  $\alpha$ -humus was precipitated and removed as usual. The analyses of the filtrates, shown in table 4, indicate that the addition of 0.15 gm. of ammonium oxalate per gram of soil extracted is sufficient to give the maximum effect. A greater amount of organic phosphorus was

found in the filtrate when the minimum amount of  $\text{HNO}_3$  required to precipitate  $\alpha$ -humus was added, but the precipitate was not well flocculated and filtration was difficult. The addition of excess acid seemed to be the more practical procedure.

A suggested interpretation of the observed behavior is that oxalate forms a complex with ferric iron, and thus, when the alkaline extract is acidified to precipitate  $\alpha$ -humus, the precipitation of ferric phytate along with the  $\alpha$ -humus is hindered.

#### *Separation of organic phosphorus by the oxalate method*

Advantage was taken of the influence of oxalate ion in separating organic phosphorus from 500-gm. samples of the  $A_1$  horizon of the Halliday podzol. After leaching with  $\text{HCl}$ , the soil was heated overnight with 5 liters of 4 per

TABLE 4  
*Effect of different amounts of oxalate on the partition of organic phosphorus from soil 7*  
Weights of P per 100 gm. of soil

TREATMENT	$\text{C}_2\text{O}_4(\text{NH}_4)_2^*$ ADDED	TOTAL P	INORGANIC P	ORGANIC P	ORGANIC P AS PER CENT OF ORGANIC P OF $\text{NH}_4$ EXTRACT
	gm.	mgm.	mgm.	mgm.	
1	...	38.0	20.1	17.9	19.2
2†	0.3	77.5	20.6	56.9	61.0
3	0.3	72.0	22.0	50.0	53.7
4	1.0	72.5	23.3	49.2	52.9
5	0.1	63.5	20.9	42.6	45.7

\* Added to 100 cc. ammonia extract (equivalent to 2 gm. soil).

† Acidified just sufficiently to precipitate  $\alpha$ -humus. Excess acid was added in all other treatments.

cent  $\text{NH}_4\text{OH}$ , in a closed vessel on a steam bath, maintaining a temperature of about  $75^\circ\text{C}$ . in the mixture. The soil residue was removed, excess  $\text{NH}_4\text{OH}$  was evaporated from the extract, and 75 gm. of  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  was added before precipitation of  $\alpha$ -humus with  $\text{HNO}_3$ . The filtrate was concentrated, and organic phosphorus was precipitated as usual by the addition of excess acid and alcohol. The product was dried in a vacuum desiccator over  $\text{H}_2\text{SO}_4$ . The results obtained in three experiments are shown in table 5.

In these experiments the hot ammonia extraction brought into solution 78–92 per cent of the organic phosphorus. The proportion remaining in the  $\alpha$ -humus filtrate was remarkably constant, 52–57 per cent of that present in the ammonia extract. A small amount of organic phosphorus remained in the alcoholic filtrate. In experiments G and F the precipitates were collected 1 hour after the addition of alcohol, and it was observed that a small amount of precipitate formed subsequently in the filtrates. After the addition of alcohol the preparations should be allowed to stand for several hours before filtering,

as was done in experiment H. The precipitate obtained in experiment F was grossly contaminated with salts thrown down by the alcohol. This result brings out the importance of removing the excess ammonia completely.

The distribution of the organic phosphorus in the various fractions obtained is given in table 6. Only 3 per cent was removed in the acid preleaching, and 3-6 per cent was lost in the final alcohol solution. About 6-20 per cent re-

TABLE 5  
*Results obtained in the separation of organic phosphorus*

EXPERIMENT.....	H	G	F
Ammonia extract			
Organic P per 100 gm. soil.....mgm.	100.8	85.3	92.0
Per cent of soil organic P.....	92.0	78.0	84.0
Inorganic P per 100 gm. soil.....mgm.	10.8	13.9	13.2
$\alpha$ -Humus			
Organic P per 100 gm. soil.....mgm.	57.4	44.5	47.8
Per cent of organic P of $\text{NH}_3$ extract.....	56.9	52.2	52.0
Inorganic P per 100 gm. soil.....mgm.	9.9	14.3	14.9
Alcoholic filtrate			
Organic P per 100 gm. soil.....mgm.	3.4	7.0	7.0
Inorganic P per 100 gm. soil.....mgm.	9.2	12.9	14.3
"Nucleotide" precipitate			
Weight.....gm.	6.1389	5.7878	11.6527
Organic P content.....per cent	4.05	3.25	1.62
Organic P per 100 gm. soil.....mgm.	49.7	38.6	38.8
Per cent of organic P of $\text{NH}_3$ extract.....	49.3	45.2	42.2
Per cent of soil organic P.....	46.0	35.1	35.3

TABLE 6  
*Distribution of organic phosphorus in various fractions*  
Percentages of total soil organic phosphorus

EXPERIMENT	ACID EXTRACT	SOIL RESIDUE*	ALCOHOLIC FILTRATE	$\alpha$ -HUMUS	SEPARATED	TOTAL
H.....	3.0	6.2	3.1	39.8	46.6	98.7
G.....	3.0	21.7	6.3	37.5	35.1	103.6
F.....	3.0	15.0	6.3	40.5	35.3	100.1

\* Includes also the organic phosphorus decomposed by hot ammonia, if any.

mained in the soil residue; this fraction includes also any organic phosphorus which may have been decomposed by hot  $\text{NH}_4\text{OH}$ , Smith (29) having found indications that a small fraction is labile to boiling ammonia. About 40 per cent remained with the  $\alpha$ -humus precipitate, and from 35-46 per cent was actually separated. This yield compares very favorably with the yields of 5-13 per cent formerly obtained without the use of oxalate. Table 6 brings



out the fact that none of the treatments caused any appreciable decomposition of the organic phosphorus, all of which is accounted for by the methods of analysis that have been devised.

The claim of Wrenshall and McKibbin to have obtained 65 per cent of the organic phosphorus from a muck soil was based on the organic phosphorus content of the cold  $\text{NH}_4\text{OH}$  extract, as determined by the method of Potter and Benton (22). In view of the demonstrated inefficiency of cold  $\text{NH}_4\text{OH}$  as an extractant, it is probable that their product represented a smaller proportion of the total organic phosphorus of the soil.

Nucleotides would be extracted from soil by hot  $\text{NH}_4\text{OH}$ , as also would phytin, provided calcium was first removed. The specific effect of calcium on the precipitation of organic phosphorus from the ammonia extract, and the striking effect produced by the introduction of oxalate ion, may be explained on the basis of the presence of phytin. The use of hot solutions in the extraction procedure was originally avoided, since it was thought that destruction of organic phosphorus compounds would occur. The completeness of extraction obtained with hot ammonia shows that this effect is small, the organic phosphorus compounds present being very stable. The product is probably a mixture of nucleotide substances and phytin.

It has been observed in this laboratory that a part of the soil organic phosphorus is stable to treatment with boiling 5 per cent  $\text{NaOH}$  (29). It was thought that there might be a difference between the organic phosphorus in the nucleotide fraction and that remaining in the  $\alpha$ -humus precipitate which could be detected in this way, and accordingly, the stability to alkaline hydrolysis of the organic phosphorus in these fractions was compared. No significant differences were found in the stability of the organic phosphorus in the  $\alpha$ -humus precipitate, the filtrate, and the nucleotide preparation, all being hydrolyzed to the extent of about 5-25 per cent. The results were rather variable but showed that these fractions contained a large proportion of alkali-stable organic phosphorus. This was regarded as further indication of the presence of phytin, which is known to be highly resistant to alkaline hydrolysis (21). As far as this test showed, the material separated was representative of the organic phosphorus of the soil.

#### SUMMARY

In an investigation of the efficiencies of various procedures for the extraction of organic phosphorus, it was found that hot 5 per cent ammonium hydroxide, preceded by leaching with  $N$   $\text{HCl}$ , gave almost complete extraction.

It was found with the majority of soils that only about 10 per cent of the organic phosphorus passed into the filtrate from  $\alpha$ -humus. The presence of oxalate ion caused the organic phosphorus of the filtrate to be increased to as much as 52 per cent.

By the use of these modifications, yields of 35 to 46 per cent of the total soil organic phosphorus were obtained as the "nucleotide" fraction, compared

with former yields of 5 to 13 per cent. The  $\alpha$ -humus still contained about 40 per cent of the organic phosphorus.

Evidence for the presence in soil of phytin as well as nucleotidic substances was seen in the behavior of the organic phosphorus of soil toward the extraction and separation treatments.

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## BOOKS

*The Biochemistry of Symbiotic Nitrogen Fixation.* By PERRY W. WILSON. The University of Wisconsin Press, Madison, Wisconsin, 1940. Pp. 302, illus. 34. Price, \$3.50.

This monograph is a sequel to *The Root Nodule Bacteria and Leguminous Plants*, by E. B. Fred and associates, published in 1932. But the book is complete in itself, in that it includes a history of the many discoveries relating to nitrogen fixation from the days of ancient Greece and Rome down to date. Recent advances in our knowledge of the chemistry of this process are reported in detail. In no other publication has this subject been so thoroughly and completely reviewed. Every agronomist will find this book highly interesting and very useful.

*Handbook of Chemistry and Physics.* Twenty-fourth Edition. Edited by CHARLES D. HODGMAN AND HARRY N. HOLMES. Chemical Rubber Publishing Company, Cleveland, 1940. Pp. 2564. Price, \$3.50.

In the enlarged 1940-41 edition of this standard work, the physical constants of organic compounds have been changed from paragraph to tabular form. An entirely new 65-page table of physical constants of industrial organic compounds has been added. Another new table deals with induced radioactivities. The mathematical, physical-constant, chemical, heat and hygrometry, and quantity and unit tables make this handbook an indispensable item on the shelves of every chemist and physicist.

*Proceedings of American Association for the Advancement of Science.* By the Association, 1940. Pp. 1109. Price, \$4.

A brief history of the association, from its founding in 1848 to 1940; its present organization; a summarized proceedings for the period from January, 1934, to January, 1940; and a directory of members, as of July 1, 1940.

*Soil Physics.* By L. D. BAVER. John Wiley and Sons, New York, 1940. Pp. 370, figs. 70. Price, \$4.

A much-needed textbook dealing with modern aspects of soil physics. The material is attractively presented. Every soil scientist will want a copy of this book on his desk for frequent reference.

*The Soils of Palestine.* By A. REIFENBERG. Translated by C. L. Whittler. Thomas Murby and Co., London, 1938. Pp. 131, illus. 10.

A discussion of the geology and climate of the Holy Land, and of the re-

sulting soils, together with some practical applications in relation to the agriculture of the area. A history of the Zionist movement and an estimate of what may be expected of this movement are appended.

*Statistical Methods.* Third Edition. By GEORGE W. SNEDECOR. The Iowa State College Press, Ames, Iowa, 1940. Pp. 422, figs. 14. Price, \$3.75.

A textbook designed to be of use to agricultural scientists who are not especially trained in mathematics. A new chapter on the design and analysis of samplings has been added. The subject is well and understandably presented.

THE EDITORS

## PALESTINE PEAT IN RELATION TO OTHER PEATS

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Palestine has, north of Lake Huleh, a considerable peat deposit, which differs in many respects from other known peat accumulations. The swampy character of the Huleh District results from an eruption of basalt which impedes the complete drainage of the Jordan into the Sea of Galilee. The resultant swamp covers an area of about 5,200 hectares. The vegetation consists chiefly of papyrus (*Cyperus papyrus*), but it also comprises ferns, reeds, and other aquatic plants (1, 3). In the surroundings of the peat profile described in table 1, the plant cover was composed mainly of Papyrus, *Phragmites communis*, Polygonum, *Bidens tripartita*, and *Cynanchum acutum*. The profile was dug in June, 1937, about a hundred meters north of the lake near the Jordan River.

### CHEMICAL CHARACTERISTICS OF PALESTINE PEAT

The distribution of organic matter and of certain inorganic components and the hydrogen-ion concentration of the peat profile are given in table 2. This table shows that peat proper is first encountered at the depth of layer E, where the water table occurs. Palestine peat contains a high percentage of mineral matter, a feature common to all lowmoor peats. The overlying layers apparently consist partly of alluvial soil, and although rich in organic matter they nevertheless contain a large quantity of mineral matter. The reaction of these soil layers is fairly alkaline, whereas the peat itself is approximately neutral and in layer F even slightly acid. The pH values correspond roughly to the amounts of calcium carbonate present. The amount of hygroscopic water is, of course, smallest in those layers (C and D) which contain the least organic matter. It will be noted that in the bottom layer (I) a much lower content of organic matter and a correspondingly higher percentage of ash as compared with the immediately overlying layers are again encountered. As pointed out in table 1, this layer contains many particles of gray clay. Such an accumulation of mineral matter in the lowest layer of a lowmoor-peat profile is common (9, p. 276).

In order to ascertain the composition of the mineral matter in the peat, an average sample of layers E-I was analyzed. The results, in percentages, were as follows: H<sub>2</sub>O, 13.95; organic matter, 62.91; SiO<sub>2</sub>, 4.22; Al<sub>2</sub>O<sub>3</sub>, 0.15; Fe<sub>2</sub>O<sub>3</sub>, 5.31; CaO, 6.99; MgO, 0.17; Na<sub>2</sub>O, 1.34; K<sub>2</sub>O, 0.24; CO<sub>2</sub>, 1.37; SO<sub>4</sub>, 3.10; P<sub>2</sub>O<sub>5</sub>, 0.15.

Most of the Huleh peat layers, like all lowmoor peats, are rich in calcium, which is present partly as carbonate and partly as sulfate. The latter is common in phragmites peat. The high content of iron is noteworthy. The peat is relatively rich in phosphoric acid and potash, but it is not so high in nitrogen as the more common lowmoor peats.

TABLE 1  
*Description of a Palestine peat profile*

HORI- ZON	DEPTH	CHARACTERISTICS
	cm.	
A	0- 10	Brownish-black clay soil with much organic matter (roots, etc.).
B	10- 20	
C	20- 40	Brown clay soil with thin roots and yellowish-red concretions of iron.
D	40- 50	Brown clay soil.
E	50- 70	<i>Water table.</i> Black peat with many decomposed roots.
F	70- 90	Black peat. Abundant brown plant residues.
G	90-110	Black peat as above. Many red moldered stains. Strong smell of H <sub>2</sub> S.
H	110-130	Black peat as above. Fewer red stains. Weak smell of H <sub>2</sub> S.
I	130-150	Black peat with many particles of gray clay. Yellowish-brown remains of plants. Few red stains. Faint smell of H <sub>2</sub> S.

TABLE 2  
*Water, CaCO<sub>3</sub>, ash, organic matter, and hydrogen-ion concentration in the peat profile*  
On an air-dry basis

HORIZON	H <sub>2</sub> O	CaCO <sub>3</sub> *	ASH†	ORGANIC MATTER‡	pH
	per cent	per cent	per cent	per cent	
A	13.10	15.23	58.38	21.81	7.7
B	13.86	14.71	63.67	25.99	7.9
C	9.40	22.13	63.81	17.04	7.9
D	8.53	24.70	62.73	17.86	7.8
E	16.78	2.83	29.87	52.10	7.1
F	16.34	0.07	21.09	62.54	6.2
G	20.04	1.23	13.09	66.33	7.3
H	19.58	4.61	17.01	61.38	7.3
I	16.39	9.32	33.86	45.64	7.3

\* Calculated from CO<sub>2</sub>. † Excluding CO<sub>2</sub>. ‡ Nitrogen content 1.53 per cent.

Clearly, the most important for our study are the organic constituents. Both the peat profile and the two main peat-forming plants were subjected to a detailed analysis according to the method of Waksman and Stevens (6). The results of this analysis<sup>1</sup> are given in table 3. When the plant materials

<sup>1</sup> Such terms as "hemicelluloses" and "cellulose" have been retained for the sake of convenience; it is fully realized, however, that the hydrolyzable fractions need not accurately represent these constituents.

and the peat products are compared, it is readily seen from this table that important changes have taken place. The ether-soluble fraction (ethereal and fatty oils, part of the waxlike and resinlike substances), the alcohol-soluble fraction (phlobaphenes, waxes, resins, alkaloids, chlorophyll, etc.), and the water-soluble fraction (sugars, organic and amino acids, alcohols, soluble proteins, part of the starches, tannins, and pectins, etc.) are greatly decreased in the peat, as are also the hemicellulose and cellulose fractions. Since the uppermost layers contain much alluvial material and since all the layers are very rich in salts, percentage increases in the lignins and proteins, calculated on a water- and ash-free basis, are very pronounced. Results calculated on this basis are shown in table 4. It will be seen that the decreases of hemicelluloses and cellulose are slightly greater in the real peat layers than in the

TABLE 3  
Composition of peat-forming plants and of peat materials  
Percentages of total dry matter

	ETHER-SOLUBLE FRACTION	ALCOHOL-SOLUBLE FRACTION	WATER-SOLUBLE FRACTION	HEMICELLULOSES	CELLULOSE	LIGNIN	PROTEINS	ASH
<i>Plants</i>								
<i>Cyperus papyrus</i> . . . .	1.43	3.08	14.39	21.35	25.74	12.15	9.49	4.53
<i>Phragmites communis</i> . .	2.45	2.67	17.59	21.26	23.45	9.59	10.62	10.95
<i>Peat</i>								
A. . . . .	0.18	0.15	1.45	2.04	0.92	13.23	4.88	74.72
B. . . . .	0.19	0.20	1.05	2.01	1.02	13.23	4.86	69.86
C. . . . .	0.16	0.11	1.34	1.94	1.01	6.24	3.13	81.19
D. . . . .	0.11	0.10	0.91	1.92	0.95	5.36	2.93	80.49
E. . . . .	0.30	0.51	1.18	4.43	1.96	33.68	10.16	37.39
F. . . . .	0.27	0.66	1.49	4.60	2.76	45.14	10.18	25.22
G. . . . .	0.23	0.81	1.48	4.64	3.39	49.11	9.21	17.01
H. . . . .	0.35	0.81	1.49	4.58	2.12	49.73	9.54	23.68
I. . . . .	0.25	0.78	1.52	3.96	1.44	34.23	7.42	45.45

alluvial layers. The corresponding accumulation of lignins is to be ascribed to their resistance to decomposition. The decrease in water-soluble material is less in the uppermost layers (especially C and D) than in the true peat layers and, correspondingly, the increase in lignin is not quite so great here. The remarkable increase in the protein fraction in all layers may be regarded as due to the synthesis of nitrogenous microbial cell substances, resistant to rapid decomposition, as shown by Waksman and Stevens (7). These nitrogenous cell substances seem, nevertheless, to be subject to decomposition under anaerobic conditions, since the percentage of the protein fraction<sup>2</sup> diminishes gradually from top to bottom.

The distribution of nitrogen both in peat-forming plants and in the peat

<sup>2</sup> The "protein fraction" does not, of course, necessarily consist of true proteins.



profile is shown in table 5. As in all peats (6), the water-soluble nitrogen fraction (ammonia, nitrate, amino acids, soluble proteins) is very low. This

TABLE 4  
Chemical composition of peat-forming plants and of peat materials  
Percentages of water-free and ash-free material

	WATER-SOLU- BLE FRACTION	HEMICELLU- LOSES	CELLULOSE	LIGNIN	PROTEINS
<i>Plants</i>					
<i>Cyperus papyrus</i> . . . . .	16.42	24.37	29.37	18.37	10.83
<i>Phragmites communis</i> . . . . .	20.11	24.25	26.75	10.93	12.12
<i>Peat</i>					
A. . . . .	6.35	8.93	4.03	57.90	21.35
B. . . . .	4.65	8.91	4.52	58.65	21.54
C. . . . .	9.62	13.93	7.25	44.79	22.47
D. . . . .	7.41	15.63	7.74	43.65	23.86
E. . . . .	2.26	8.48	3.76	64.48	19.46
F. . . . .	2.29	7.07	4.24	69.34	15.63
G. . . . .	2.15	6.73	4.29	71.32	13.38
H. . . . .	2.17	6.67	3.08	72.49	13.90
I. . . . .	3.07	7.99	2.90	69.01	14.96

TABLE 5  
Nitrogen distribution in peat-forming plants and in peat materials  
Percentages of total nitrogen

	WATER-SOLUBLE	HYDROLYZABLE BY DILUTE ACIDS	NONHYDROLYZABLE BY DILUTE ACIDS ("HUMIN" N)
<i>Plants</i>			
<i>Cyperus papyrus</i> . . . . .	9.9	25.7	64.4
<i>Phragmites communis</i> . . . . .	10.5	25.0	64.5
<i>Peat</i>			
A. . . . .	4.7	28.1	67.2
B. . . . .	4.4	27.6	68.2
C. . . . .	4.4	22.0	73.6
D. . . . .	5.3	24.1	70.6
E. . . . .	3.7	24.8	71.5
F. . . . .	3.7	34.5	61.8
G. . . . .	4.2	20.5	75.3
H. . . . .	4.0	22.6	73.4
I. . . . .	4.8	14.6	80.6

is largely true also of the nitrogen hydrolyzable by dilute acids (true proteins). Greenhouse experiments on the availability of nitrogen in Huleh peat carried out by us have yielded negative results, a finding obviously attributable to

the relative insolubility of the greater part of the nitrogen. The amount of insoluble "humins" nitrogen present is rather high, but is normal when compared with other types of lowmoor peat. The percentage of water-soluble nitrogen is much higher in the living plant than in the peat. Similar observations were made by Waksman and Stevens (7), who ascribe the relative decrease of soluble nitrogen in the peat partly to assimilation by growing plants and partly to the action of microorganisms which decompose the hemicelluloses and cellulose. In fact, all our figures point to the secondary formation of insoluble proteins, as demonstrated by Waksman and others (2, 5).

TABLE 6  
*Composition of various plants which give rise to peat*  
Percentages of total dry matter

	PLANT	ETHER-SOLUBLE	ALCOHOL-SOLUBLE	WATER-SOLUBLE	HEMICELLULOSES	CELLULOSE	LIGNINS	PROTEINS	ASH
Typical for lowmoor peats	Cladium*	1.14		6.87	21.45	28.31	29.09	7.19	3.89
	Carex*	2.54		12.56	18.36	28.20	21.08	7.08	3.30
	<i>Cyperus papyrus</i>	1.43	3.08	14.39	21.35	25.74	12.15	9.49	4.53
	<i>Phragmites communis</i>	2.45	2.67	17.59	21.26	23.45	9.59	10.62	10.95
Typical for highmoor peats	Sphagnum*	1.47		3.86	30.82	21.13	6.97	5.88	3.18
	Sphagnum†	1.94	2.97	6.02	21.91	28.57	18.81	4.05	5.10
	Sphagnum†	2.54	2.23	10.11	26.79	28.69	16.20	2.73	3.24

\* According to Waksman and Stevens (7).

† According to Kivinen (2) and Waksman (8).

#### PALESTINE PEAT IN COMPARISON WITH OTHER PEATS

The chemical and physicochemical properties of a peat are closely linked with the nature of the plant associations from which the peat is derived. The nature of these plant associations depends on climatic conditions and on such ecological factors as the chemical composition of the water. If only the two principal peat types are taken into account, it may be said that, in general, waters rich in calcium and other nutrients favor the development of sedges and reeds and ultimately give rise to a lowmoor peat; on the other hand, telluric waters or waters originating from soils poor in soluble minerals favor the development of mosses and bring about the formation of a highmoor peat.

Table 6 shows, however, that the chemical composition of different plant species is not so specific as might theoretically be supposed; such differences as are actually found may, in many cases, be explained by differences in development, age, and ecological conditions (2). The main difference apparent from these figures is in the protein content, which seems to be much lower in mosses than in sedges or reeds. Nevertheless, the corresponding peats show

much more marked differences, and these must be ascribed to differences in the ecological conditions of the locality concerned, which in turn are largely determined by climatic factors.

Just as differences in climate may cause the same parent rock to yield essentially different soil types, so differences in climatic and ecological conditions may cause plants of similar composition to yield essentially different peat types. On the other hand, it must be borne in mind that, just as the nature of the parent material has a certain effect on soil formation, so also the nature of the plant exerts a considerable influence on the resulting peat. The nitrogen content of the plant is particularly important in this connection.

A lowmoor peat is characterized mainly by a low content of cellulose and a relatively high content of lignins, proteins, and ash; a highmoor peat, on the

TABLE 7  
*Nitrogen, mineral content, and pH of various peats*  
On the basis of total dry matter

TYPE OF PEAT	PLANT FORMATION	NITROGEN	PHOSPHORIC ACID	POTASSIUM	CALCIUM OXIDE	pH
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Highmoor* . . . . .	Sphagnum	0.64-0.74	0.03-0.04	0.02-0.03	0.15-0.65	3.5-4.5
Lowmoor* . . . . .	Carex	2.47-2.94	0.14-0.20	0.05-0.06	1.80-4.50	5.5-6.5
	Phragmites	2.29-3.32	0.09-0.28	0.09-0.19	1.80-4.50	5.5-6.5
Palestine† . . . . .	<i>Cyperus papyrus</i> and <i>Phragmites communis</i>	1.53	0.15	0.24	6.99	7.1

\* According to Waksman (9, pp. 264, 276).    † Average sample.

other hand, is poorer in the latter constituents and richer in the former. Consequently, lowmoor peats consist of much better decomposed plant residues, their percentage of lignin, the end-product of decomposition, being greatly increased. At the same time, synthesis of proteins by microbic cell activity takes place. Highmoor peats have a much lower percentage of proteins, and because, in turn, greater proportions of hemicelluloses and cellulose are still intact, the physicochemical qualities of these peats are very different from those of lowmoor peats.

Tables 7 and 8 illustrate the differences between peats and indicate the relative position of Palestine peat, a product of the Mediterranean climate. The amount of mineral matter in Palestine peat is extraordinarily high and must be ascribed to the prevailing climatic conditions. As the climate is Mediterranean, a rainy winter season alternates with a completely dry period in summer (4). Under these conditions the water table is subject to frequent

fluctuations. The upper layers of the bog may dry out in summer and thus cause a capillary water movement from the lower layers to the surface. In consequence, salts are not leached out but concentrate in the uppermost layers, which also increase in lime content. Because the salts are not leached out, Palestine peat is rich in phosphoric acid and potash, nutrients which are important for bacterial activity. Closely associated with the high content of calcium in most layers is the hydrogen-ion concentration, which is near the neutral point and lower than that in lowmoor peats generally. In consequence, organic compounds in Palestine peat are saturated by bases. To this peculiarity, the high degree of decomposition in this peat must be partly ascribed, since a slightly alkaline reaction is most favorable to bacterial activity. The drying up of the top layers favors aerobic processes, and these too cause de-

TABLE 8  
*Organic composition of various peats*  
Percentages of total dry matter

TYPE OF PEAT	PLANT FORMATION	ETHER-SOLUBLE	ALCOHOL-SOLUBLE	WATER-SOLUBLE	HEMI-CELLULOSES	CELLULOSE	LIGNIN	PROTEINS	ASH
Highmoor	Sphagnum*	3.96			16.24	19.91	38.26	6.58	1.50
	Sphagnum†	3.53	4.56	7.82	18.15	16.55	38.53	3.81	1.48
Lowmoor	Phragmites and Cyperaceae*	1.10		1.24	8.95	0	50.33	18.72	10.13
	Cyperaceae†	4.73	5.10	2.98	12.32	5.44	41.78	14.72	4.95
Palestine	<i>Cyperus papyrus</i> ‡ and Phragmites	0.28	0.71	1.43	4.44	2.33	42.38	9.30	29.75

\* Waksman (8, p. 187).

† Kivinen (2, p. 22).

‡ Average of layers E-I.

composition processes to proceed much more intensively. The high temperature prevailing throughout the year likewise favors bacterial processes.

Table 8 shows the organic composition of Palestine peat in relation to other types of peat. From this, it may be seen that, compared with other lowmoor peats, Palestine peat possesses a relatively low amount of proteins. This circumstance might be ascribed to the presence of calcium, which causes nitrogen to be liberated and thus counteracts the synthesis of proteins by bacteria. The figures for cellulose and hemicelluloses in Palestine peat are very characteristic: because of the prevailing high degree of decomposition they are much lower than the figures for these products in other lowmoor peats. The same is true also of the ether- and alcohol-soluble substances, their great resistance to decomposition notwithstanding. It is only because of the high ash content that the increase in lignin is not pronounced (cf. table 4, where all figures are calculated on an ash-free basis).

## CONCLUSIONS

Palestine peat is a lowmoor peat, characterized by a high ash content. This wealth of mineral matter is due to the Mediterranean climate, which causes the drying out of the uppermost layers in summer and a consequent accumulation of salts in the peat. Sufficient amounts of nitrogen, potash, phosphoric acid, and calcium to enable strong bacterial activity are present.

Because of the relatively high content of calcium, the peat is saturated with bases and possesses a neutral to slightly alkaline reaction in most layers.

Decomposition is very advanced because of high temperature, the pH reaction, and the presence of nutrients which favor bacterial activity and because of aerobic conditions which prevail in the uppermost layers during summer.

The high degree of decomposition causes a great increase in lignin, on the one hand, and a very pronounced decrease in hemicelluloses, cellulose, waxes, and fats, on the other. As in all lowmoor peats, a synthesis of proteins has occurred, although to less than the usual extent. This may be due to the neutral to slightly alkaline reaction, which favors the decomposition of nitrogenous substances and thus offsets protein formation.

Differences in the chemical composition of various types of peat are, in general, more marked than the differences in the composition of their corresponding plant sources. The former differences must be ascribed, therefore, not so much to the particular chemical character of the plant formations as to the prevailing ecological differences, which in turn are dependent on climatic factors.

A high degree of decomposition, coupled with a high ash content, accounts for particular physical and physicochemical properties of the Huleh peat.

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## A NEW SEDIMENTATION TUBE FOR ANALYZING WATER-STABLE SOIL AGGREGATES

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The productive value of a soil is commonly dependent upon its structure (3, 5, 6). A current problem in soil science is to devise apparatus and procedures to measure and to specify soil structure quantitatively. The most important of the features that require measurement are known, but present apparatus and procedures are still inadequate to give the necessary information, and their development is in a state of flux. Russell (4) lists three main types of investigations that have been used for the specification of soil structure; namely, determinations of permeability, of porosity, and of aggregate size distribution.

The size distribution of soil aggregates is fundamental to the description of a soil under specific conditions, and a knowledge of the stability of these aggregates in water is a material aid to the prediction of the structural behavior of the soil under the influence of varying conditions, such as changes in moisture content, temperature, and porosity.

The water-stable aggregate size distribution may be determined with the sedimentation tube developed by Cole and Edlefsen (1), the size ranges of the aggregates being expressed in terms of settling velocity in water and not convertible to dimensions of mass or length. This method has given good results in this laboratory and elsewhere (3). It is not suited to routine soil structure studies, however, because of the time required to make an analysis. Field structure, even in small experimental plots, is so variable that a large number of analyses must be made in order to obtain statistically reliable results. The structure of the soil is also transitory and may change in samples during storage. One of the requirements, then, of an apparatus for measuring the aggregation of field soils, is that it lend itself to rapid determinations. This paper describes a sedimentation tube and technic for making water-stable aggregate analyses which are comparable to those of Cole and Edlefsen and which can be made in approximately one-tenth the time. A minimum of 2 days is required to make a single analysis by the Cole and Edlefsen procedure, whereas four to five analyses can be made daily by the use of the modified tube and technic described in this paper.

### THE MODIFIED SEDIMENTATION TUBE

The modified tube (pl. 1, fig. 1) is similar in form to that of Cole and Edlefsen, consisting of an outside tube 36 inches long with a segmented liner of the

same length made up of 2-inch segments machined to fit. The only drainage outlets in the Cole and Edlefsen tube are small screw holes in the end caps. For drainage, the modified tube has brass blocks  $\frac{3}{4}$  inch in diameter and  $\frac{3}{8}$  inch high soldered on the outside of the outer tube at 2-inch centers along its axis, thus providing a block opposite the longitudinal center of each segment of the liner. Holes are provided, such that when the tube is in a horizontal position with the blocks upright, a vertical hole extends through each block, the outer tube, and through the liner at the center of each segment. The blocks are tapped to receive  $\frac{1}{8}$ -inch pipe plugs.

The only other difference between the modified tube and that of Cole and Edlefsen is the means used to secure the caps which close the ends. Instead of threaded caps and tube ends, the caps are secured by wing nuts threaded on lugs which are soldered to the tube.

In addition to the sedimentation tube, an arrangement of siphons to control drainage of the tube is used. An individual siphon is provided for each segment, with these individuals so interconnected as to enable easy control of the over-all drainage rate and likewise to ensure the same rate from each segment. The arrangement and the operation of the siphoning device are made clear in plate 1 and in the explanation of the technic for making a water-stable soil aggregate analysis.

The tube is assembled for filling by putting on the bottom end cap, inserting the liner segments, and screwing the pipe plugs into the brass blocks on the side. The tube is filled to near the top with distilled water before the soil, slaked in a beaker outside the tube, is poured into it. After this partial filling, the top cap with the petcock removed is placed on the tube, and the temperature of the water is recorded from a thermometer inserted through this cap. The tube is then completely filled with water and the petcock screwed into the cap. It is now ready for mixing and sedimentation, which are conducted according to the procedure of Cole and Edlefsen.

After mixing and sedimentation, the tube is carefully removed from the wheel and placed on the siphoning rack, the plugs are removed, and a powdered flocculating agent, hydrated potassium alum, is introduced into the suspension through the holes in the side. The flocculation produced causes rapid settling of the suspension and allows rapid drainage without altering the axial distribution of the sediments.

Immediately after the introduction of the flocculating agent, siphon tubes filled with water are lowered vertically through the holes in the sedimentation tube until they touch the bottom of the liner segments. After the interval allowed for flocculation and settling, the siphons are started by progressively lowering the siphon bottles to such an elevation that the water level in the bottles after the tube is fully siphoned will be slightly below the bottom of the sedimentation tube. An average flocculation and settling time of 30 minutes, and a drainage time of 1 hour were satisfactory for the soils used.

The siphon bottles, one for each siphon, are closed with two-hole rubber stoppers pierced by two tubing leads, the siphon lead and the air outlet lead. The siphon leads are always submerged in the bottles, extend through the stoppers, and connect through rubber tubing to the siphon tubes. The air outlet leads extend just into the tops of the bottles and are connected to a manifold which has its outlet in a bubble tube leading to near the bottom of a large barostat bottle shown at the left in figure 3, plate 1. The water level in this bottle is controlled and may be gradually lowered by slow drainage. The elevation of the water in the barostat bottle determines the air pressure that must be attained before air can escape from the bubble tube. This control of the air pressure and the difference between the water elevation in the sedimentation tube and the bottles control the rate of siphoning. After

TABLE 1

*Mean percentages and standard errors of means of Yolo loam, with settling velocities equal to or less than definite values, as measured by the Cole and Edlefsen and by the modified sedimentation tube method*

MAXIMUM SETTLING VELOCITY  cm./sec.	COLE AND EDLEFSEN		MODIFIED TUBE	
	Mean percentage	S.E.	Mean percentage	S.E.
0.2	35.5	0.31	35.6	0.30
0.4	41.0	0.27	40.8	0.31
0.6	46.8	0.40	45.7	0.27
0.8	51.1	0.45	52.0	0.38
1.0	56.5	0.43	57.6	0.36
1.2	60.5	0.39	61.6	0.31
1.4	66.5	0.41	65.2	0.27
1.6	70.5	0.32	69.5	0.25
1.8	74.5	0.31	73.6	0.26

the siphon bottles have been lowered to their final position, siphoning is continued to completion by progressively lowering the water level in the barostat.

The results of aggregate analyses obtained by the Cole and Edlefsen tube without flocculation and those obtained with the modified tube by the technic described in this paper are compared in table 1. Seven analyses were made with each tube on a sample of air-dry Yolo loam.

The density of the suspension in the two lowest segments obviously affected the settling velocities through these segments. Results from the bottom three segments were not utilized in calculating the settling velocity distribution. Thus, approximately 74 cm. of a 36-inch tube was available for particle differentiation. Dependent upon the temperature of the water in the tube and for settling velocities corrected for water at 20°C., this length of tube will measure settling velocities of 2.1 to 2.5 cm. per second in the temperature range of 26 to 19°C.



## DISCUSSION

The analyses made with this tube and technic do not cover the full range of aggregate size normally found in field soil. Most of the analyses in this laboratory have been made on Yolo loam in which 20 to 40 per cent by weight of the soil was in aggregates with settling velocities greater than 2.0 cm. per second. Results from the use of this tube in our laboratory confirm the conclusion of other workers (2), that the summation percentages of the aggregate sizes measurable by the sedimentation tube are inversely related to aggregation; i.e., a low summation percentage indicates a well-aggregated soil and vice versa.

There are several possibilities for extending the size range of aggregates that may be determined by the sedimentation tube method; using a longer tube, making analyses at lower temperatures, or using a liquid of greater viscosity than water. Aggregation however, may vary with temperature and may be altered by a liquid other than water. It would probably be practicable to use a tube as long as 5 feet. A 5-foot tube, using a 30-second sedimentation time, would measure aggregates with settling velocities of 4.0 cm. per second.

## SUMMARY

The importance of soil structure in soil evaluation, and the present state of knowledge and means for its quantitative specification, render the development of apparatus and procedures for such specification a fundamental current problem in soil physics. The size distribution of water-stable aggregates is an important criterion in the solution of this problem.

The normal variation and dynamic character of soil structure in the field and the inadvisability of long-time storage of soil samples for later study, require that structural analysis be made rapidly.

A sedimentation tube and technic are described, by which the number of aggregate distribution determinations that may be made in a given time is increased tenfold over that formerly possible by the sedimentation method.

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PLATE

## PLATE 1

## THE SEDIMENTATION TUBE AND THE SIPHONING DEVICE

FIG. 1. Sedimentation tube with the end caps secured by threaded lugs and wing nuts. The siphon holes at the left end of the tube are closed with pipe plugs.

FIG. 2. Rear view, showing the siphon bottles in the background in the raised position preliminary to siphoning. The siphon tubes, also in the raised position, are ready to be inserted into the sedimentation tube.

FIG. 3. Front view of the tube and siphoning device: 1. siphon bottles lowered to the final siphoning position; 2. the sedimentation tube; 3. rack, adjustable vertically, supporting the siphon tubes which are inserted in the sedimentation tube; 4. a segment of the liner. An aspirator bottle at the left functions as a barostat and is shown connected to the manifold through which air escapes from the siphon bottles.

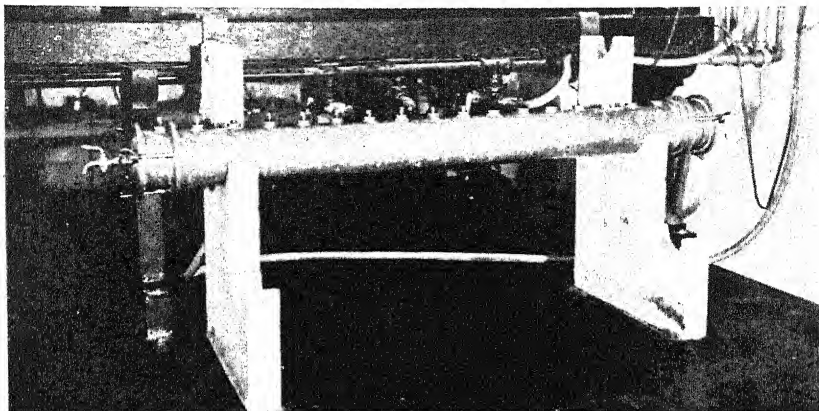


FIG. 1

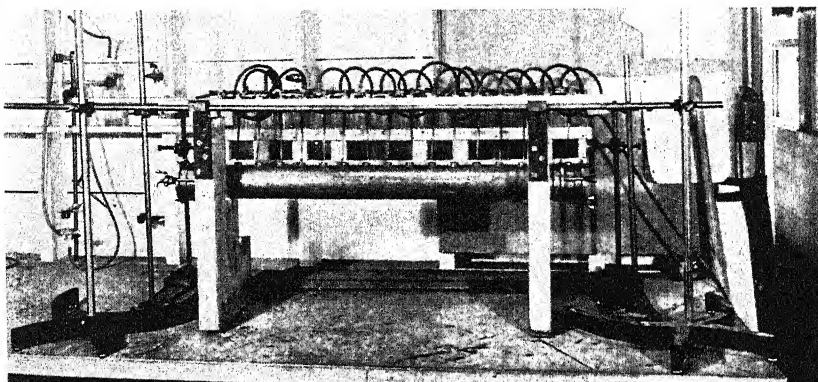


FIG. 2

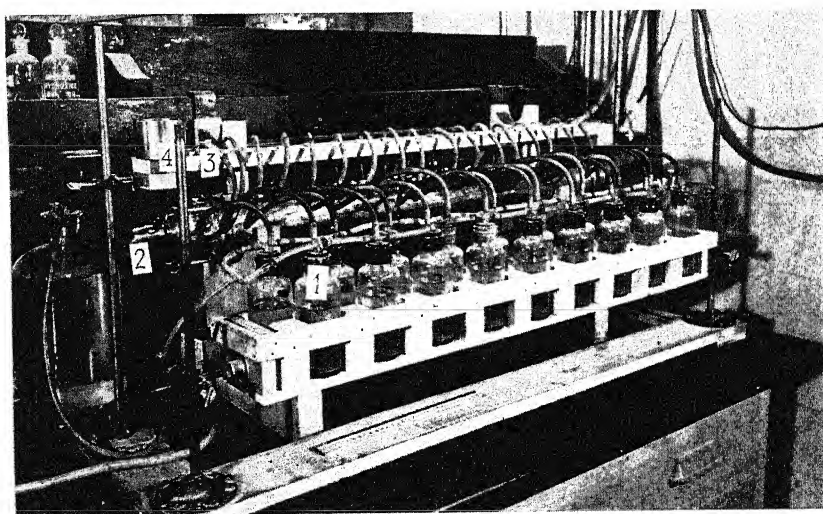


FIG. 3



# CHANGES IN SOIL AGGREGATION IN RELATION TO BACTERIAL NUMBERS, HYDROGEN-ION CONCENTRATION, AND LENGTH OF TIME SOIL WAS KEPT MOIST<sup>1</sup>

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The favorable influence of soil microorganisms upon the formation of water-stable aggregates of soil particles has been reported by several investigators (2, 3, 4, 6, 9, 11). Close scrutiny of these reports reveals, however, that only qualitative relationships have been established. Changes in bacterial numbers in some instances have been assumed, rather than determined. In an effort to evaluate the role played by bacteria in changes in the degree of aggregation of soils of the prairie group, the experiments herein reported were carried out.

## METHODS

Two soil samples, both Geary silt loam from the college agronomy farm, taken to the depth of 7 inches were used. Soil I was from a rotation series started in 1926. The rotation consists of 2 years of sweet clover, kafir, oats, and wheat. All crop residues have been returned to the plats. The area was limed in 1926 but has received no fertilizer or manure treatment. The soil for the first experiment was taken on May 25 from the plat that had been planted to kafir a few days earlier; the sample for the second experiment was taken on August 23 from a plat that had been plowed in preparation for wheat. Soil II was taken from an area on a field where erosion has been rather severe. Manure has been regularly applied at the equivalent rate of approximately 2 tons per acre annually. No lime has been applied, but an occasional application of superphosphate to wheat has been made. The crops during recent years have consisted principally of sorghums, oats, and wheat. The sample for the first experiment was taken from a field previously planted to Atlas sorgo; the second sample was taken from an adjoining area that had been plowed for wheat, following oats. Sampling dates correspond with those given for soil I.

The chemical and physical characteristics of the two soils are indicated in part by the analyses given in table 1.

<sup>1</sup> Contribution No. 312, department of agronomy, and Contribution No. 201, department of bacteriology.

<sup>2</sup> Suggestions concerning the statistical study of the data were made by H. H. Laude.

Mechanical analysis, moisture equivalent, and pH were determined, respectively, by means of the Bouyoucos hydrometer, the centrifuge, and the glass electrode. The nitrate nitrogen was determined by the phenoldisulfonic acid method, and the total nitrogen was measured by digesting the samples according to the Gunning-Hibbard procedure and distilling the ammonia into a boric acid solution.

In the first experiment both samples were put through a 4-mesh screen. For the second experiment the samples were put through a 4-mm. U. S. standard sieve. After air-drying in the laboratory, each soil was thoroughly mixed, and samples containing 50 gm. of oven-dry soil were separated for the experiment and put into covered Petri dishes. The treatments used are recorded for each experiment. All amendments were applied in solution of such concentrations as to provide the proper amounts of both water and amendment. An equal amount of distilled water was added to the untreated samples. In the first experiment 15 cc. of solution was added to each sample, but in the second experiment only 14 cc. was used. To samples selected at random the materials were applied from a burette by allowing the liquid

TABLE 1  
*Characteristics of soils used in the study*

SOIL	MECHANICAL ANALYSIS			MOISTURE EQUIVALENT	pH	NITRATE NITROGEN	TOTAL NITROGEN
	Sand	Silt	Clay				
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>p.p.m.</i>	<i>per cent</i>
I	37.5	31.0	31.5	21.8	6.1	90.4	0.136
II	39.0	31.0	30.0	21.5	5.7	30.6	0.118

to drip down the side of the Petri dish as it was rotated slowly. All samples were incubated at 28°C. The degree of aggregation and the bacterial numbers were determined at three different periods, and at any given period three of the samples were removed at random for aggregate study and two were removed for bacterial counts.

The percentage aggregation was determined by wet sieving, using 0.105-, 2.0-, and 4.0-mm. U. S. standard sieves. These were moved vertically through a distance of about 2 inches at the rate of 30 strokes per minute in distilled water, changed after each washing. The samples were put through a 2-mesh wire screen and wet sieved without drying and without the previous addition of water. The data recorded for each treatment represent an average of the three individual readings.

The bacterial numbers were determined by means of plate counts using egg albumin agar.<sup>3</sup> Quadruplicate platings were made on each of the two samples taken for bacterial counts, and the number reported represents an average of the eight counts.

<sup>3</sup> Agar-agar, 20 gm.; glucose, 1 gm.; MgSO<sub>4</sub>, 0.25 gm.; K<sub>2</sub>HPO<sub>4</sub>, 0.50 gm.; FeSO<sub>4</sub>, trace; yeast extract, 0.50 gm.; and powdered egg albumin, 0.25 gm. per liter adjusted to a neutral reaction with NaOH (pH 7).

Statistical analyses were made according to the methods outlined by Snedecor (10).

## RESULTS

### *First experiment*

In the preliminary experiment, in addition to the untreated samples, samples of each of the two soils were treated with 500 p.p.m. calcium nitrate and with 1000 p.p.m. sucrose. No water was added during the time of incubation. Samples were analyzed after three different periods of incubation. The results for soils I and II were very similar as judged from both aggregate analysis and bacterial count. Analysis of variance indicated that the treatments did not result in a significant difference in aggregation on either the 1-day or the 18-day interval although the ratio of the mean squares (between treatments divided by the within treatments) or *F* values for the 18-day period did approach sig-

TABLE 2

*Influence of calcium nitrate and sucrose on the percentage of soil in aggregates larger than 0.105 mm. and the relation of bacterial numbers to aggregation*

TREATMENT	AVERAGE OF SOILS I AND II					
	Aggregates larger than 0.105 mm.			Bacterial numbers per gram of dry soil		
	1 day <i>N</i> = 6	8 days <i>N</i> = 6	18 days <i>N</i> = 6	0 days <i>N</i> = 16	8 days <i>N</i> = 16	18 days <i>N</i> = 16
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>millions</i>	<i>millions</i>	<i>millions</i>
Untreated.....	75.2	79.2	74.7	0.1	24.5	17.5
Calcium nitrate, 500 p.p.m.....	77.1	80.5	77.0	0.3	27.0	29.0
Sucrose, 1000 p.p.m.....	78.1	86.0	84.0	1.2	56.0	46.0
Ratio of mean squares, <i>F</i> value...	1.86	9.00**	4.50*			

\* Significant.

\*\* Highly significant.

Correlation coefficient for the relationship of bacterial numbers to percentage aggregation using the paired readings making up these averages for all treatments on the 8- and 18-day periods was 0.758, which is highly significant.

nificance for both soils. For the eighth day, differences in aggregation resulting from treatment were significant. Because of the similarity in the response of the two soils to treatments only average figures are presented in table 2.

By combining the data for the two soils the level of significance of the difference in aggregation resulting from treatment was raised somewhat. For the 1-day incubation the differences were not significant, which substantiates previous findings (8) that neither calcium nitrate nor sucrose at the rates used influences directly the degree of aggregation of soils. For the 8-day incubation period the differences were highly significant, but for 18 days the differences were only significant. It should also be noted that the maximum aggregation occurred on the eighth day.

A statistical study, using analysis of variance, was also made of the aggre-



gates larger than 2.0 and 4.0 mm., but significant differences were not obtained for either soil at any period studied for either of the two size groups. The ratios of the mean squares (between treatments divided by the within treatments) or *F* values were about the same for each soil for any one incubation period but lower than those obtained by using total aggregates larger than 0.105 mm. Therefore only summations of results for aggregates retained on all three screens are presented.

When the average bacterial number and the average percentage aggregation for all treatments after 8 and 18 days' incubation were paired the correlation coefficient between the two variables was found to be 0.758, which is highly significant (10). The 1-day results were not paired for the correlation coefficient study because the bacterial numbers were determined as soon as possible after the treatments, whereas the aggregate analyses were made 1 day later. The highly significant correlation coefficient suggests the important role of bacteria in changes in the degree of aggregation but gives no indication as to whether their role is direct through the organisms themselves binding the soil particles together, or whether their importance is due to an indirect influence, namely, the formation of decomposition products that are more effective as cementing agents than is the added organic matter.

### *Second experiment*

In the second experiment soils I and II were each given the following treatments: 500 p.p.m. calcium nitrate, 1000 p.p.m. sucrose plus 500 p.p.m. calcium nitrate, 10,000 p.p.m. sucrose plus 500 p.p.m. calcium nitrate, 1000 p.p.m. peptone, and 10,000 p.p.m. peptone; samples of each soil also were left untreated. Readings were made at the end of 1, 4, 8, 13, 19, and 47 days. The moisture content of the soil was kept up by the addition of distilled water as needed. The samples treated with 10,000 p.p.m. of either sucrose or peptone required more frequent additions of water than did the other samples. The experimental findings together with the results of the statistical study are recorded in table 3.

The percentages of aggregates coarser than both 2 mm. and 4 mm. were available, but because of the lower variability in the summation figures only the results for the aggregates larger than 0.105 mm. are presented.

The percentage of aggregates larger than 0.105 mm. increased as a result of incubation, and in 9 out of 12 cases reached the peak on the eighth day. In two instances the maximum was reached on the fourth day and in the other case on the thirteenth day. Except for a few irregularities there was a continuous decline in the percentage of the sample aggregated on each sampling date following the peak. After 47 days, three of the soil I and four of the soil II samples had returned to a level of aggregation approximating that of untreated soils on the first analysis date.

The over-all relationship between bacterial numbers and percentage aggregation for the 72 pairs of data including all treatments at all dates on both soils

TABLE 3  
Relationship of number of bacteria to the degree of aggregation of soils

TREATMENT	SOIL	1 DAY		4 DAYS		8 DAYS		13 DAYS		19 DAYS		47 DAYS		CORRELATION COEFFICIENT, ALL DAYS, BOTH SOILS, $N = 12$
		A	B	A	B	A	B	A	B	A	B	A	B	
Untreated	I	68.9	23	71.7	21	75.8	14	74.1	20	72.1	32	74.8	30	0.227
	II	71.1	10	76.0	11	73.8	11	73.4	26	73.1	45	67.2	14	
Calcium nitrate, 500 p.p.m.	I	73.0	37	73.1	30	79.9	22	75.5	25	76.7	37	75.2	65	0.155
	II	67.6	14	67.9	12	74.1	10	72.4	19	72.3	65	67.9	43	
Peptone, 10,000 p.p.m.	I	86.5	65	90.7	2000	91.6	436	90.3	420	86.3	428	88.3	48	0.377
	II	82.2	1200	87.0	1400	89.4	284	87.7	264	85.4	2300	79.0	64	
Peptone, 1000 p.p.m.	I	79.3	10	81.5	73	88.0	44	81.6	64	83.0	64	81.8	61	0.026
	II	73.9	116	81.1	48	81.5	26	82.8	50	80.0	92	67.3	19	
Sucrose, 10,000 p.p.m. + calcium nitrate, 500 p.p.m.	I	72.3	416	89.7	564	94.9	184	92.5	316	90.5	169	89.8	134	-0.214
	II	82.9	448	88.0	272	94.5	158	93.8	91	92.4	195	82.3	28	
Sucrose, 1000 p.p.m. + calcium nitrate, 500 p.p.m.	I	76.8	173	79.9	184	81.5	55	79.7	56	78.2	103	73.1	106	-0.101
	II	67.8	192	76.2	128	72.8	73	75.1	53	69.6	155	70.5	40	
Correlation coefficient, all treatments, $N = 12$	Both soils	0.407		0.677*		0.689*		0.746**		0.326		0.535		0.639** $N = 72$

A: The percentage of the sample in aggregates larger than 0.105 mm.

B: Bacterial numbers as millions per gram of dry soil.

\* Significant.

\*\* Highly significant.

is indicated by the correlation coefficient of 0.639, which is highly significant. This is in close agreement with the results obtained in the first experiment. For the individual incubation periods of 1, 4, 8, 13, 19, and 47 days, the correlation coefficients for the relationship are 0.407, 0.677 (significant), 0.689 (significant) 0.746 (highly significant), 0.326, and 0.535, respectively. When any one treatment for the two soils for all sampling dates is considered, it is found that the correlation coefficient for the relationship is much below the level of significance. For both of the sucrose treatments the relationship is indicated by nonsignificant negative values. By excluding the results obtained on the forty-seventh day, the correlation coefficients for the two peptone treatments, the two sucrose treatments, and the untreated samples were found to be nonsignificant negative values.

The addition of water to the samples previous to analysis usually resulted in an increase in bacterial numbers and a decrease in the percentage aggregation. That the additional water was not the most important factor in causing this decrease in aggregation is indicated by the similarity in the aggregation trends in both the first and the second experiments. In the first experiment no water was added during the course of the incubation, whereas in the second, water was added at intervals as previously noted. Great variation in the moisture content of the samples was never allowed. The added water may have contributed to the increased bacterial numbers.

It is also important to note that for the samples treated with organic substances, except for 10,000 p.p.m. of peptone on soil II, the maximum bacterial numbers were recorded after either 1 or 4 days' incubation. In no instance does the maximum bacterial population correspond to the maximum percentage aggregation. There is a distinct tendency for the degree of aggregation to lag behind the change in the bacterial numbers; this would explain the low positive or negative correlation coefficient for each treatment on all days where the numbers tend to be of the same general magnitude, i.e., either very high or very low. This would suggest that the bacteria play principally an indirect role in aggregate formation. The products of microbial activity appear to be the fundamental factor involved in aggregation, the organisms functioning chiefly by transforming the added organic matter into a more active cementing material.

In order to show that bacteria play an indirect role in soil aggregation, calculations of the actual weight of the cells as organic matter were made. If each cell is assumed to occupy 1 cu.  $\mu$ , all the living cells produced in the highest count obtained with 10,000 p.p.m. of peptone, would occupy approximately 0.25 per cent of the total weight of the soil. If this is calculated as dry weight, the cells produced would be only approximately 0.025 per cent of the soil or would increase the organic matter content of the soil less than 1 per cent. This is further indication that the influence of bacteria on soil aggregation is probably indirect.

It has been demonstrated that partly decomposed organic matter influences

aggregation directly, the extent of the influence depending to a certain degree upon the quantity of organic matter and partly upon its quality. In the light of this information and of the results obtained in this investigation, it appears that the several studies in the past relating to the effect of soil bacteria on aggregation merely serve to report an acceptance of the long-established fact that the bacteria of the soil are capable of transforming added organic matter either by partial decomposition or by synthesis, with the consequent effects by these on soil structure.

#### *Effect of hydrogen-ion concentration*

The decomposition of added organic substances resulted in changes in the hydrogen-ion concentration of the soil. For any one treatment the pH was approximately constant throughout the period of study. The sucrose treatments caused a slight lowering of the pH, whereas the peptone treatments resulted in a marked increase in the pH. Changes in hydrogen-ion concentration could not be related to changes in the percentage aggregation of the samples.

In a supplementary experiment using soil I of the second experiment, the effect of changes in hydrogen-ion concentration upon aggregation was studied. Untreated samples and samples treated with 10,000 p.p.m. sucrose plus 1000 p.p.m. calcium nitrate were the only groups studied. Fifteen 50-gm. samples, to which were added 16 cc. of solution, were prepared for each group. After incubation at 28°C. for 8 days the hydrogen-ion concentration for each of the two groups was adjusted to three levels by addition of 50 cc. of saturated solution of calcium hydroxide, which was equivalent to 2.1 tons of calcium carbonate per acre; 50 cc. of 0.1 *N* HCl, which was the equivalent acidity of 5 tons of calcium carbonate per acre; and 50 cc. of distilled water to each of five samples, two of which were used for pH measurements, leaving three for aggregate analysis. The solutions were allowed to remain in contact with the soil for exactly 3 hours, after which aggregate analysis was made. The excess liquid was poured off the remaining samples for pH measurement, and these were left in the laboratory for 3 days, during which evaporation brought them to approximately field moisture capacity, as indicated by their appearance.

The reaction was adjusted at the end of the incubation period instead of at the beginning, in order to measure the direct influence of changes in hydrogen-ion concentration on the effectiveness of any cementing material produced by the organisms. Variations in reaction at the beginning of the incubation period might have resulted in altering the quantity of cement produced. The results are presented in table 4.

The marked increase in aggregation in the sucrose-treated samples suggests the presence of a considerable excess of biological cementing material over that probably present in the soil which did not receive the sucrose.

Analysis of variance of the aggregate data indicates that within neither the sucrose-treated nor the untreated samples were significant differences between

amendments obtained. The ratios of the mean squares (between treatments divided by the within treatments) were 0.98 and 1.01, respectively, for the sucrose and the untreated samples. For a 5 per cent level of significance, 3.68 is necessary. From these results it appears that the gums, mucilages, or other materials that may be responsible for the increased aggregation in the sucrose-treated samples are no more effective as cements in the presence of calcium than in the presence of an acid. This would suggest that lime, through its influence on organic matter, is not a direct factor in the stability of aggregates where the comparison is between two acid systems, one of which contains more calcium. This is in substantial agreement with previously reported studies (7) in which it was shown that calcium organic colloid was no more

TABLE 4

*Influence of change in hydrogen-ion concentration after incubation on the effectiveness of biological aggregate cement*

TREATMENT	AGGREGATES LARGER THAN 0.105 MM.	pH	CALCIUM CARBONATE EQUIVALENT ADDED
	<i>per cent</i>		<i>tons/A.</i>
10,000 p.p.m. sucrose plus 1000 p.p.m. calcium nitrate			
a. Distilled water.....	85.5	5.8	0.0
b. Calcium hydroxide.....	87.1	6.8	2.1
c. Hydrochloric acid.....	88.3	4.1	-5.0*
Ratio of mean squares, <i>F</i> value.....	0.98†		
Untreated			
a. Distilled water.....	68.7	6.0	0.0
b. Calcium hydroxide.....	71.3	6.7	2.1
c. Hydrochloric acid.....	68.6	4.1	-5.0*
Ratio of mean squares, <i>F</i> value.....	1.01†		

\* Equivalent acidity.

† Nonsignificant.

effective and usually was less effective than hydrogen organic colloid in the formation of water-stable aggregates. It might be expected that calcium could indirectly influence aggregation as a result of microbial activity, either by stimulating the activities of the same groups of microbes or by altering the species of organisms developing in the soil. Such results might be expected to occur where the lime is added at the beginning of the incubation period. Aggregation studies under such conditions would reveal the over-all direct and indirect effects of lime.

*Relation of length of time soil was kept moist to degree of aggregation*

Aggregate analyses were made on the air-dry samples of soils I and II used in the second experiment. The average results for three samples from each soil revealed that only 49.2 and 40.8 per cent respectively of the water-stable

aggregates of soils I and II were larger than 0.105 mm., in contrast to 68.9 and 71.7 per cent respectively for the same soil after 1 day in the moist state.

The influence of time on the degree of water-stable aggregation of moist soils, originally put through a screen and air dried, is indicated in figure 1 where the average percentage aggregation for soils I and II is plotted against time in days. These findings conflict with the results presented by Kolodny and Joffe (5) but are in essential agreement with those reported by Cole (1) for soils irrigated after cultivation.

Aggregate analyses were repeated on the same air-dry soils in order to verify the accuracy of the results with the technic employed. The average of three readings gave values of 45.8 and 43.8 per cent larger than 0.105 mm. for soils I and II respectively, which is in close agreement with previous results. Likewise the average of three readings from samples of dry soils I and II used

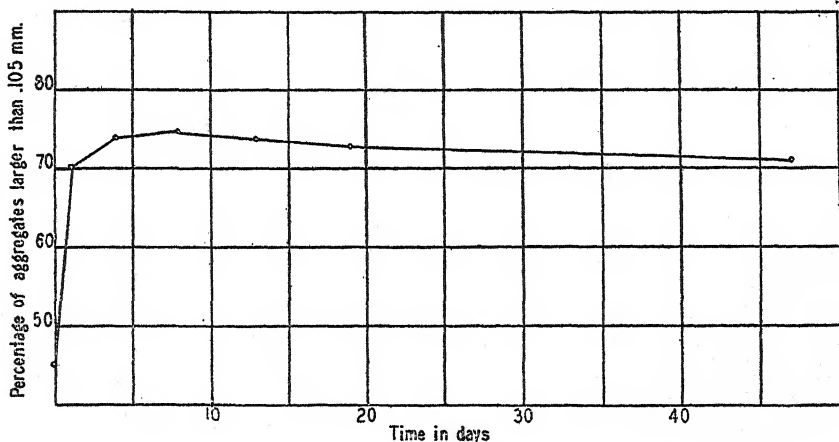


FIG. 1. CHANGES IN AGGREGATION RESULTING FROM MOISTENING AN AIR-DRY SOIL AND KEEPING IT AT CONSTANT MOISTURE FOR VARYING LENGTHS OF TIME

in the first experiment gave values of 55.1 and 48.9 per cent for aggregates larger than 0.105 mm., or an average for both soils of 52.0 per cent in contrast with 75.2 per cent for the same soils after 1 day of incubation in the moist state, as shown in table 2.

That this condition is not peculiar to the Geary soil is indicated by results obtained with surface samples of Cherokee silt loam employed in another experiment. In this experiment the samples were moistened and kept in the laboratory for varying lengths of time. The average of three measurements on the air-dry sample and the average of nine readings for three different dates on samples untreated but kept moist in the laboratory for several days gave 33.4 and 66.0 per cent respectively of aggregates larger than 0.105 mm. The reason for the sharp increase in the percentage of aggregates during the first 24 hours following the addition of water to the air-dry samples is not evident from the results obtained in any of the experiments.

Though the initial bacterial numbers were not determined at the beginning of the second experiment, such information is available for the first experiment as reported in table 2. The numbers in the air-dry samples were low. The bacterial population probably increased manyfold during the initial 24 hours as indicated by the bacterial numbers reported for the first day in table 3. Such growth would probably have resulted in some change in the soil organic matter and in the production of limited quantities of gums or mucilaginous materials even in the untreated soil. If the initial aggregation is assumed to be the same for all treatments, it is evident from the results presented in table 3 that bacterial numbers are not directly proportional to the increase in aggregation during the first day of incubation.

Further evidence that soil organisms are not directly responsible for the sharp initial increase in the degree of aggregation is furnished by an experiment conducted with soil II. The treatments in this experiment consisted

TABLE 5  
*Influence of sterilization\* on the aggregation of soil II*

TREATMENT	AGGREGATE LARGER THAN 0.105 MM. IN STERILIZED SOIL		
	1 day <i>N</i> = 3	8 days <i>N</i> = 3	18 days <i>N</i> = 3
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Untreated.....	67.1	71.5	71.0
Calcium nitrate, 500 p.p.m.....	74.5	73.8	74.9
Sucrose, 1000 p.p.m.....	73.3	73.2	73.2

\* Sterilization was complete; no bacteria could be found in any of the samples during the 18-day experiment.

of 500 p.p.m. calcium nitrate and 1000 p.p.m. sucrose, applied to air-dry samples. These and the untreated samples were sterilized immediately by means of dry heat at 170°C. After sterilization the samples were remoistened with sterile distilled water and incubated. The results are shown in table 5.

An analysis of three air-dry samples of the same soil showed that only 48.9 per cent of the sample consisted of aggregates larger than 0.105 mm. In a parallel experiment, unsterilized samples had, at the end of 1 day of incubation, 73.4, 77.7, and 78.2 per cent of aggregates larger than 0.105 mm. respectively for the untreated, 500 p.p.m. calcium nitrate, and 1000 p.p.m. sucrose samples. These values are only slightly higher than the corresponding values for the sterilized soil, thus indicating that the initial elevation in aggregation resulting from moistening air-dry soil is not due to microorganisms. These facts do not preclude the possibility of the influence of microbial decomposition products, since sterilization may have so altered the organic matter that it served the same function as the products of microbial activity. There is no evidence, however, to support this idea.

A more logical explanation appears to be that the large initial increase in aggregation is due to the rehydration and activation of the cementing materials, made partly ineffective by the crushing and dehydration resorted to in the preparation of the experimental soil.

#### SUMMARY AND CONCLUSIONS

A study was made of the relationship of bacterial numbers to the degree of aggregation in a soil of the prairie group. Varying the bacterial numbers within wide limits by means of soil treatment and length of time of incubation resulted in a highly significant over-all relationship between bacterial numbers and aggregation when all treatments and all incubation periods are considered. For different periods of incubation, significant or highly significant positive relations occur only on the fourth, eighth, and thirteenth days of incubation. No one treatment for all incubation periods gave a significant correlation coefficient for the two variables. Maximum aggregation in no instance coincided with maximum bacterial numbers but lagged behind the bacterial numbers during both the growth and the death phases.

The data indicate that bacteria are associated with and responsible for the aggregation of soil particles only in so far as they are responsible for the accumulation of certain metabolic products that function as cementing materials.

Changes in hydrogen-ion concentration as a result of the decomposition of added organic materials were not concomitant with changes in aggregation. Adjustment of the pH of soils within the approximate limits of 4.1 to 6.7, did not significantly influence the stability of preformed aggregates. No tendency was observed for calcium to improve the stability of aggregates.

The addition of water to pulverized air-dry soil caused a very sharp and rapid increase in the percentage of water-stable aggregates. This increase occurred in sterilized soils and hence is independent of microbial activity.

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# THE MEASUREMENT OF STRUCTURAL STABILITY AND PERMEABILITY AND THE INFLUENCE OF SOIL TREATMENTS UPON THESE PROPERTIES<sup>1</sup>

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Although a general knowledge of and an interest in soil structure have occupied a definite place in the advancement of soil science, there has been a renewed interest in the practical application of soil physics within recent years. This is due partly to a clearer understanding of what constitutes structure and structural stability and to a recognition of the great importance that permeability plays in water absorption, runoff and erosion, and possibly soil respiration. It has been shown that the soil mantle is a tremendous reservoir for water and that any reasonable precipitation can be accommodated by almost any soil, provided such precipitation can enter as fast as it occurs. It can also be shown that among cultivated soils, in the majority of instances, the first few centimeters of the exposed surface is the least absorptive layer. Virgin forested or grassland soils normally possess a good structure and permeability in the upper horizons.

Soils brought under cultivation, particularly if the cropping systems are such that the surface is not covered for a considerable proportion of the time, are gradually subjected to forces which tend to break down those natural structural aggregates which may have persisted for centuries under natural conditions.

Among the structure- and permeability-destroying forces which operate upon soils under cultivation, the following may be mentioned: impact of beating rain drops upon exposed aggregates; shearing, polishing action of plowshares and other implements; leaching of soluble salts from upper layers during long fallow periods, resulting in dispersion of aggregates surrounded

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with a solution of low osmotic concentration; absence for long periods, at or near the surface, of organic residues to serve as food for macro and micro life; destruction of vertical channels by horizontal tillage operations; compaction resulting from animals, man, or machine. These and many other forces are permitted to operate in and on soils under cultivation but are virtually non-existent in soils under natural forest or grassland conditions.

It seems desirable, therefore, that cultural practices, rotations, and cropping systems be examined with a view of determining whether, and to what degree, each system or practice tends to destroy or repair structure. Doubtless much of the phenomenon known as soil depletion may be explained as directly or indirectly due to destruction of permeability. It is recognized that a long time may be required to produce any permanent alteration in the aggregate condition of a soil; hence it seems desirable to study present conditions in soils the history or treatment of which is known, rather than to set up an experiment designed to determine such structural changes which might not produce significant alterations for a quarter to a half century.

#### REVIEW OF LITERATURE

The literature contains many evidences of attempts to determine quantitatively the characteristics and extent of structural modification. The results of field measurements conducted upon soils *in situ* (12, 13, 15, 16, 22, 23, 24, 25, 28) reflect significant physical effects as manifested by permeability, soil resistance, density, etc. Laboratory procedures (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 17, 18, 20, 21, 30, 31, 34) differ in principle, somewhat, from the field tests. Essentially these determinations constitute an attempt at the quantitative characterization of the primary physical units of the soil, namely, the individual particles and the role of aggregation or structural formation and maintenance.

#### METHODS

This paper deals with the examination of different methods for measuring structural stability and the adoption of a satisfactory procedure, and the application of this procedure in the study of experimental plot soils which have been under known treatment and cropping plans for many years.

The physical condition which determines infiltration and percolation rate is related to total pore space, size of pore, noncapillary porosity, volume weight, swelling capacity, dispersion ratio, and state of aggregation. Immediately following any tillage operation a soil may appear to be in good tilth. But how persistent is this condition? Is it destroyed by one or two rains, or does the soil retain its absorptive capacity even after several rains? If its granules are of sufficient size and stability, it will retain its absorptive capacity, but if they are readily dispersed, surface runoff will take place. To obtain a measure of the size and stability of the natural soil aggregates, the following method is proposed.

### *Aggregate analysis*

The air-dried sample is passed through a 4-mm. sieve with a minimum of mechanical abrasion. A quantity equivalent to 50 gm. of air-dried soil is carefully weighed out and placed in a large beaker with 200 cc. of distilled water and allowed to slake for 12 hours. It is then transferred to a 1-liter graduated cylinder, made up to volume with distilled water, inverted end over end twenty times, and set in upright position. A 25-cc. pipette is immediately inserted so that its point is immersed to a depth of 18 cm., and within 5 seconds 25 cc. is withdrawn and placed in a beaker to be evaporated. The pipette is rinsed with distilled water. This aliquot contains the fine sand, silt, and clay, i.e., material finer than 0.2 mm.

This method of obtaining the aggregates of fine sand size by pipetting differs from the wet sieving method, but in comparative trials between the two, agreement was close. The replicates of the pipetted fraction, however, were more consistently concordant.

Aliquots of the silt and clay fractions are withdrawn with the pipette after settling intervals as outlined by Wright (33), although it is recognized that an aggregate will fall at a slower rate than a discrete particle of the same diameter. These are evaporated, weighed, and the percentages calculated.

Aliquots of the separates smaller than 0.2 mm. in diameter having been removed, the coarser separates are obtained by wet sieving as follows:

Decant a large portion of the supernatant turbid liquid and pour the remainder upon a nest of sieves having aperture diameters of 0.2 and 2.0 mm. Standard screens of 70 and 10 meshes per linear inch have apertures of these sizes. A gentle stream of water is applied to the material on each screen to wash off adhering particles; the content of each screen is dried and weighed. The quantity of the various separates is calculated as a percentage on the water-free soil basis.

In this work the gravel separate, having primary particle or aggregate diameter limits ranging from 2 to 4 mm., is carefully determined. It is believed that soil constituents of this size often occur and are of great importance physically.

### *Mechanical analysis*

The mechanical analysis was made by a modification of the pipette method (26) as follows: An amount equivalent to 25 gm. of water-free 4-mm. soil is put into a large beaker and 200 cc. of distilled water is added; the contents of the beaker are boiled, cooled, and 10 cc. of normal sodium carbonate is added. Transfer to a stirring apparatus and stir for 15 minutes to effect complete deflocculation. Transfer the contents of the dispersion cup to a 1-liter graduated cylinder, make up to the liter mark, and after stirring well, pipette off the various sized separates as described under aggregate analysis. Follow this with the wet sieving as described, and calculate the percentage of each separate including the gravel, 2.0 to 4.0 mm.

*Stability index*

The results of the mechanical and aggregate analyses as described may be plotted as in figure 1 and 2. The aggregate analysis will contain a larger

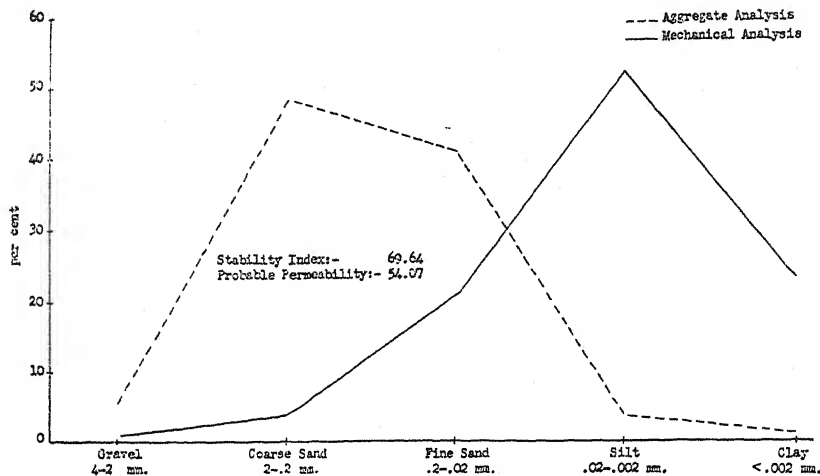


FIG. 1. PHYSICAL ANALYSIS DATA CURVES FOR UNDISTURBED HAGERSTOWN SILT LOAM; SITE—BLUEGRASS SOD

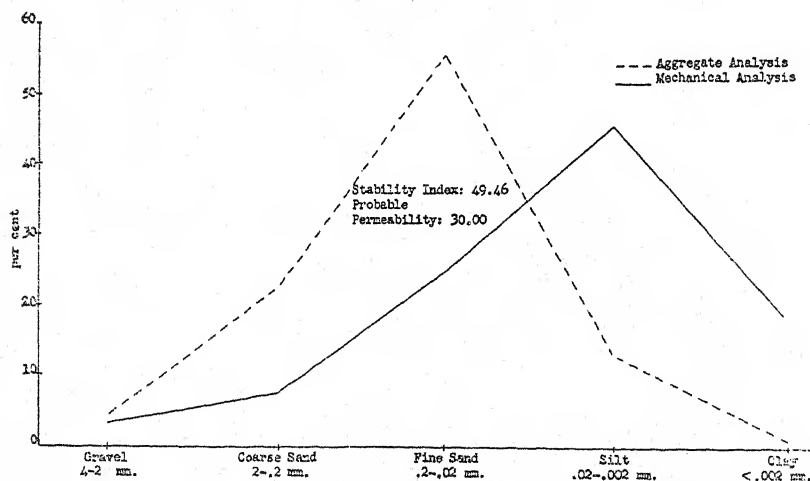


FIG. 2. PHYSICAL ANALYSIS DATA CURVES FOR CULTIVATED, UNFERTILIZED HAGERSTOWN SILT LOAM; SITE:—JORDAN SOIL FERTILITY EXPERIMENT, TIER 1, PLOT 14

percentage of the coarser separates and a smaller percentage of the finer separates than will the complete mechanical analysis. In some soils, having very unstable or easily dispersed aggregates, the results obtained by both methods will be nearly the same, whereas with other soils, having stabilized

granules, the aggregate analysis gives a much larger percentage of the coarser sized separates. This is a measure of granule stability and may be expressed by a single figure which is designated as the "stability index." The stability index is the sum of the positive differences between the aggregate analysis and the complete mechanical analysis, that is, all the differences to the left of the point where the two curves intersect (figs. 1 and 2). Obviously the larger the stability index, the more stable is the structure of the soil. It is therefore a useful way of determining whether or not a soil will remain absorptive after several rains and may be used to determine whether or not a given cultural practice has built up or destroyed structure.

The stability index may be expressed as:

$$S = \Sigma a - \Sigma m$$

in which

$S$  = stability index

$\Sigma a$  = sum of aggregate analysis percentages to the left of the intersection.

$\Sigma m$  = sum of mechanical analysis percentages to the left of the intersection.

#### *Probable permeability*

Granules may be stable but so small that the intervening pores are inadequate to allow significant amounts of gravitational water to flow between them. The probable permeability of cultivated soil is determined to a large degree by the amount of coarse units, whether they be primary particles or stable aggregates. In percolation studies, flow is very slow through soils the units, particles, or aggregates of which are smaller than 0.2 mm. in diameter. Consequently, the sum of all the percentages of stable aggregates and particles greater than 0.2 mm. has been considered as a measure of the probable permeability. For this value an aggregate analysis alone is sufficient. It is expressed as:

$$P = \Sigma a'$$

in which

$P$  = probable permeability

$\Sigma a'$  = sum of aggregate analysis percentages for particles greater than 0.2 mm.

The reliability of this expression for probable permeability may be shown by the fact that the correlation coefficient between the probable permeability as determined by the preceding calculation and the actual percolation rate determined in percolation tubes was +.81 and +.91 for the Jordan and truck crop plot soils respectively.

The application of the method of aggregate analysis and calculation of the stability index and probable permeability for a sample of soil in excellent structure obtained from under a bluegrass sod of long standing, is shown in

table 1 and in figure 1. Though this soil on complete mechanical analysis is shown to be high in silt and clay, two separates which produce impermeability, the aggregate analysis reveals the presence of a large percentage of aggregates of the dimensions of coarse sand and fine sand. This soil allows rapid percolation continuously. Its stability index is 69.64, and its probable permeability, 54.07. These values may be compared with those obtained on the unfertilized Jordan plot shown in figure 2. The latter plot had a stability index of 49.46 and a probable permeability of 30.00. This method was used in the comparisons which follow.

#### *Volume weight*

While each of the individual soil samples was being taken, its volume was also obtained. A metal sampling cylinder of known volume was driven into the soil to a depth corresponding to that of the surface soil. The entire contents were removed, air dried, weighed, and the volume weight calculated with the data therefrom. Ten cores from each plot constituted the composite sample.

#### *Percolation rate*

Laboratory percolation rates were obtained by the use of half-pint glass percolation tubes the soil contents of which remained under constant hydraulic head.

#### *Organic matter content*

The organic content of the soil was determined by the chromic acid titration procedure of Schollenberger (29) as modified by Tiurin (32).

#### EXPERIMENTAL

In order to determine whether or not cropping systems and fertilizing practices have a measurable effect upon soil structure and permeability, certain plot soils at the Pennsylvania Agricultural Experiment Station were examined by the methods described.

The Jordan fertility plots have been under continuous treatment for 58 years. The soil is Hagerstown silt loam; the rotation, corn, oats, wheat and clover, fertilizers in most instances being applied to the corn and wheat only. Tier 1 has never received lime except on those plots which receive lime only or lime and manure. Tier 2 was unlimed until 1921, at which time each plot was limed according to its requirement. Further information concerning these plots may be found in numerous bulletins (27). For this study certain plots were chosen to show the effect of treatments which might be expected to produce structural changes. The treatments and analytical results are given in table 2. For comparative purposes the results obtained on a sample of bluegrass sod adjacent to the plots are also presented.

The first striking fact brought out in table 2 is that the plot soils as a group have much lower stability indexes, lower probable permeabilities and actual

TABLE 1  
*Physical analysis data for bluegrass sod soil*

ANALYSIS	GRAVEL	COARSE SAND	FINE SAND	SILT	CLAY
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Aggregate . . . . .	5.66	48.41	40.98	3.80	1.15
Mechanical . . . . .	0.98	3.93	20.50	51.76	22.83
Difference . . . . .	4.68	44.48	20.48	—47.96	—21.68
Stability Index . . . . .	69.64			—69.64	
Probable Permeability . . . . .	54.07				

TABLE 2  
*Physical values of certain selected plots of the Jordan fertilizer experiment*

TIER	PLOT	TREATMENT*	STABIL- ITY INDEX	PROB- ABLE PERMEA- BILITY	PERCO- LATION	OR- GANIC CON- TENT	VOLUME WEIGHT	PORE SPACE
					<i>cc./min.</i>	<i>per cent</i>		<i>per cent</i>
1	14	Check	49.46	30.00	4.5	2.36	1.25	50.0
2	14		47.12	27.92	2.5	2.21	1.27	49.2
1	16	6 tons manure	57.10	33.08	16.0	2.79	1.23	50.8
2	16		60.37	34.02	15.0	3.10	1.16	53.6
1	20	10 tons manure	64.66	31.63	18.0	3.26	1.17	53.2
2	20		59.69	29.08	14.0	2.67	1.20	52.0
1	17	24 lbs. dried blood, P, K	58.58	29.08	6.0	2.90	1.16	53.6
2	17		64.24	31.59	11.0	2.71	1.14	54.4
1	21	72 lbs. dried blood, P, K	64.71	28.66	10.0	2.93	1.14	54.4
2	21		58.21	28.25	4.0	2.29	1.22	51.2
1	26	24 lbs. nitrate of soda, P, K	60.82	28.06	6.3	2.65	1.16	53.6
2	26		58.23	28.97	7.8	2.72	1.21	51.6
1	28	72 lbs. nitrate of soda, P, K	55.02	23.92	5.0	2.33	1.22	51.2
2	28		56.99	23.17	4.4	2.40	1.22	51.2
1	30	24 lbs. sulfate of ammonia, P, K	57.63	22.21	3.75	2.41	1.26	49.6
2	30		60.15	31.94	11.5	2.41	1.22	51.2
1	32	72 lbs. sulfate of ammonia, P, K	57.37	20.45	4.0	2.52	1.25	50.0
2	32		60.15	22.02	6.5	2.24	1.26	49.6
1	22	6 tons manure and 2 tons burnt lime to corn	63.07	29.72	12.0	3.21	1.18	52.8
1	23	Burnt lime to corn	51.12	22.17	3.0	2.45	1.23	50.8
1	34	2 tons ground limestone	60.34	23.01	6.0	2.24	1.24	50.4
		Bluegrass sod	69.64	54.07	37.0	3.36	1.07	57.2

\* Similarly numbered plots were similarly treated except that those from tier 2 received lime in addition to the treatments indicated.



percolation rates, lower organic contents, and higher volume weights than the soil from the bluegrass sod adjacent to the plots. This sod has in all probability been in bluegrass for 50 or more years. Obviously, the rotation of corn, oats, wheat, and clover for 58 years has allowed structural breakdown, no matter what soil treatment the various plots received. Nevertheless, fertilizers, manure, and lime have had a measurable, though small, effect. The effect observed may be due partly to a direct physicochemical action and partly to the density of sods produced by the treatment. Manure, even though used in light applications has produced structural improvement, as revealed by the stability index, probable permeability, and volume weight. Lime did not always increase structural stability or permeability. Lime alone,

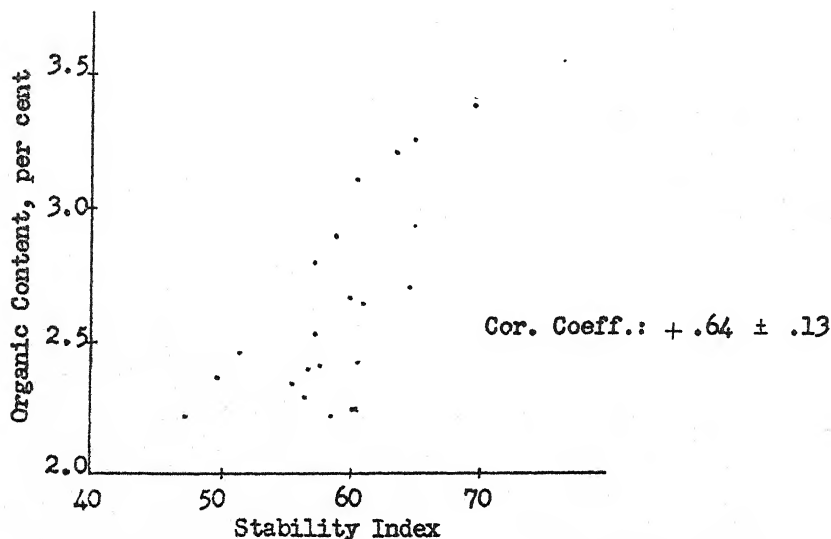


FIG. 3. RELATION BETWEEN ORGANIC CONTENT AND STABILITY INDEX, JORDAN SOIL FERTILITY EXPERIMENT

particularly burnt lime, reduced structural stability but when used in conjunction with manure it appeared to produce physical benefits. There is not much evidence that the liming in 1921 of plots which received commercial fertilizers only has had much effect upon granule stability, permeability, or percolation rate. The organic content of the different plots, which itself is determined partly by the past history and partly by the effects of fertilizer treatments, is definitely correlated with physical measurements (fig. 3).

The truck crop fertilizer experiment (19) which was laid out in 1917 on soil very similar to that of the Jordan plots and not far from the latter, afforded another excellent opportunity to study the effect of cropping systems and fertilizer treatments upon physical properties. In this experiment the rotation from 1917 to 1928 consisted of cabbage, potatoes, tomatoes, and wheat, a cover crop of timothy and clover sown in the wheat being plowed under the

following spring. Wheat was used as a cover crop after each crop. From 1928 to 1938 corn was substituted for wheat as a main crop, and rye and vetch were substituted for wheat as a cover crop. These are definitely destructive cropping systems so far as maintaining soil structure and organic matter is concerned. It was deemed desirable to discontinue the experiment in 1938, but during the 21 years that it was in operation, the structure was so nearly destroyed that a large portion of all heavy rains was lost as runoff, and yields were poor even though fertilization was heavy. The treatments and physical data obtained on selected plots from this experiment are recorded in table 3 together with a sample from the uncompacted part of a bluegrass roadway adjacent, the latter having been in sod at least as long as the experiment lasted.

The first notable observation is the low stability index of all unmanured plots of this experiment as compared with those of the Jordan plots. The

TABLE 3

*Physical values of certain selected plots of the truck crop fertilizer experiment—section A*

TIER	PLOT	TREATMENT	STABIL- ITY INDEX	PROB- ABLE PERMEA- BILITY	PERCO- LATION	OR- GANIC CON- TENT	VOLUME WEIGHT	PORE SPACE
					cc./min.	per cent		per cent
1	13	Check	49.37	25.41	3.0	2.03	1.41	43.6
1	14	20 tons manure	56.59	26.02	15.0	3.26	1.29	48.4
1	16	40 tons manure	58.84	31.56	24.0	5.34	1.10	56.0
2	3	Nitrate of soda, P, K	49.18	18.09	4.0	1.98	1.32	47.2
3	1	Check	49.02	15.52	6.0	1.76	1.39	44.4
3	2	Sulfate of ammonia, P, K	48.46	18.86	1.2	1.97	1.34	46.4
3	3	Dried blood, P, K	48.87	19.27	1.5	1.95	1.33	46.8
6	3	Complete fertilizer, 10 tons manure	54.51	24.34	10.0	3.05	1.26	49.6
		Bluegrass sod	69.64	50.00	37.0	3.36	1.03	58.8

unmanured vegetable garden plots have indexes of from 48 to 49, whereas those of the unmanured Jordan plots range from 47 to 64. The probable permeabilities of the unmanured vegetable plots are from 15 to 25, whereas those of the unmanured Jordan plots vary from 20 to 32. These differences are doubtless the result of differences in cropping systems. The rotation of corn, oats, wheat and clover followed on the Jordan plots has maintained a better structure than the truck rotation which contained no significant soil conserving crop.

The vegetable garden plots which receive heavy applications of manure or manure and fertilizer have a structure which is visibly better as observed in the field and as revealed by the physical measurements. Ten tons of manure with fertilizer, 20 tons without, and 40 tons without, gave stability indexes of 54.5, 57, and 59 as against stability indexes of 48 to 49 for heavily fertilized plots and of 49 for the check plots. The probable permeability, organic content,

and volume weights show similar trends. The lowest probable permeability obtained on the Jordan plots was 20, and the lowest value obtained on the vegetable plots was 18. Obviously, the lack of a sod crop for a full season has permitted a deterioration of the structure on the vegetable plots. The values obtained following physical analysis of the soil developed under blue-grass sod,  $S = 70$  and  $P = 54$ , offer a striking contrast with cultivated plot soils found 3 feet away,  $S = 50$  and  $P = 30$ .

Some indication as to the probable factors producing granule stability may be obtained from correlations between stability indexes and measured values. Figures 3 and 4 show significant correlations between the stability indexes and the organic contents of plots. Correlation between probable permeability

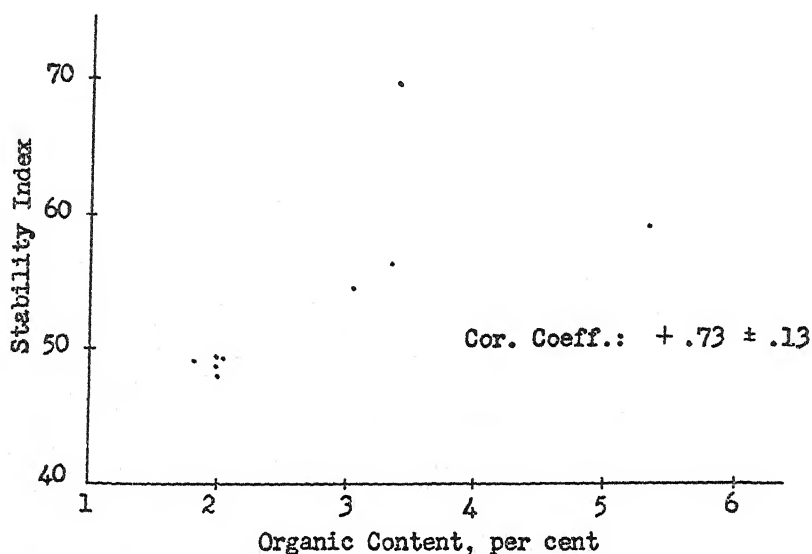


FIG. 4. RELATION BETWEEN ORGANIC CONTENT AND STABILITY INDEX, TRUCK CROP FERTILIZATION EXPERIMENT

and organic content for the Jordan plots is significant,  $+ .65$ . That for the truck crop experiment is  $+ .37$ , a nonsignificant figure. The stability index is closely correlated with the volume weight; both reveal the same properties. The coefficient of correlation between these two qualities was  $-.83$  for the Jordan plots and  $-.92$  for the vegetable crop plots. The correlation coefficients between the probable permeability indexes and volume weight are  $-.77$  and  $-.96$  for the Jordan Soil Fertility and Truck Crop Fertilization Experiments respectively.

#### SUMMARY

A method is described which is sufficiently refined to reveal the small alterations in aggregate size and stability that may be produced within a single

soil type by different soil treatments through variations in cultural practice, fertilization, erosion, etc. A numerical measure of the structural stability of the aggregates and the probable permeability of soils is presented.

When the method is used to study differences in structure and permeability produced by cropping systems, fertilizing, and liming, it was shown that on a single soil:

(a) A rotation of corn, oats, wheat and clover over a period of 58 years caused a breakdown of aggregates as compared with sod land adjacent.

(b) A vegetable cropping system including no sod crop, and with cover crops only for soil improvement, produced after 21 years a poorer structural condition than the rotation described in (a).

(c) Farm manure whenever used produced definite physical improvement.

(d) Liming did not significantly alter the structural condition.

(e) Other things being equal, structural stability is closely correlated with the organic content.

(f) The volume weight exhibits a significant inverse relationship with structural stability, probable permeability, and organic content.

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## CALCIUM SATURATION AND ANAEROBIC BACTERIA AS POSSIBLE FACTORS IN GLEIZATION<sup>1</sup>

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The agencies and chemical changes concerned in the production of the blue-gray horizon in the deeper part of the soil profile, commonly spoken of as "glei development," have provoked much discussion.<sup>3</sup> This process of gleization has regularly been associated with standing water and the consequent iron reduction to give the gray color. Calcium as a nutrient for the responsible bacteria has not been suggested, and as a contributing factor, calcium has not been segregated.

Since this horizon usually consists of a sticky, compact, and structureless clayey mass, its water retention is generally high and movement through it very slow. Its location deep in the solum would exclude influences by atmospheric oxygen but would not necessarily prohibit the infiltration from the soil surface of energy-supplying materials of organic origin. These might serve for use by anaerobic bacteria. Since recent studies emphasize the encouraging role of mineral nutrients, particularly of calcium, in organic matter decomposition and the diminished decomposition, or organic matter accumulation, under limited calcium supply,<sup>4</sup> it seems possible that gleization occurs in the zone where the three factors, ample calcium, organic matter infiltration, and standing water, all operate jointly for anaerobiosis.

That this may be true is suggested by an observation of clay standing under water. Putnam subsoil had been treated with acid and washed thoroughly to produce the resulting acid clay. Three lots in suspension were then treated separately with calcium hydroxide, magnesium hydroxide, and aluminum hydroxide. All were preserved under waterlogged conditions. After 3 months, the samples of the calcium-treated clay and of the magnesium-treated clay had developed the bluish-gray color resembling that of glei. The sample treated with aluminum showed no color change throughout its depth.<sup>5</sup>

<sup>1</sup> Contribution from the department of soils, Missouri Agricultural Experiment Station, Columbia, Missouri. Journal Series No. 701.

<sup>2</sup> Professor of soils.

<sup>3</sup> Joffe, J. S. 1936 *Pedology*, pp. 328-344. Rutgers University Press, New Brunswick, N. J.

<sup>4</sup> Albrecht, W. A. 1938 Nitrate production in soils as influenced by cropping and soil treatments. *Missouri Agr. Exp. Sta. Res. Bul.* 294.

<sup>5</sup> The differences in the samples were first observed and drawn to attention by Hans Winterkorn, research associate professor of soil mechanics, University of Missouri.

Since these clays contain some relatively stable organic matter representing the residue of microbial action which has been moved downward by podzolization, such organic residue would of necessity be relatively deficient in calcium and in magnesium as bacterial nutrients. It would still be of service as a source of microbial energy, however, and the promotion of microbial growth might occur when these mineral shortages were restored.

That an attack on this organic material by anaerobic microorganisms occurred where the calcium and magnesium were applied to the clay was indicated by the color change, and pointed to the reduction of the iron for the organic matter oxidation. Where neither calcium nor magnesium was applied to serve as nutrient bases, but where aluminum, a nonnutrient, or potassium was substituted, these anaerobic performances were not initiated.

That the exchangeable calcium plays a role is suggested by some studies by Wilde, of the Wisconsin Experiment Station.<sup>6</sup> Calculations from his determinations of exchangeable calcium in different horizons of alpha, beta, and gamma glei soils show that the glei horizon occurs where the calcium saturation of the clay was increasing in the successively deeper layers approaching the glei layer. In one of his soils, which he assigns to the Colby series, the percentage of calcium saturation in the zone of glei formation was slightly less than 50 per cent. In another, assigned by him to the Miami series, it was above this figure.

That at least 50 per cent calcium saturation of the clay should be required to foster anaerobic bacterial activity is in interesting agreement with the approximate 50 per cent calcium saturation of colloidal clay required for significant growth and nitrogen fixation by soybeans. It raises the question whether the profile horizons above that of glei formation are not simply of too low a degree of calcium saturation for microbial activity, so that the percolating organic matter is not of service until it has moved downward to the horizon of sufficient calcium saturation and the corresponding relative saturation of other bases.

These observations prompted a simple laboratory test. Untreated Putnam clay and Putnam clay saturated with different cations were taken in equal amounts and mixed with equal amounts of the humus compound extracted from a calcium-deficient soil. They were all stored under water. The soil given calcium became gray. No significant color change occurred in the clay saturated with aluminum, potassium, or hydrogen, or in the untreated clay.

These changes were noticeable in less than 4 weeks and became very distinct in 8 weeks or at the intervals of closer observation. After this latter period the stoppers were removed, cleaned, and the upper part of the cylinder cleaned in connection with the examination for odors. A distinct odor of hydrogen sulfide was detected over the calcium clay. It was less noticeable but yet present over the natural and potassium clay. There was none over the hydro-

<sup>6</sup> Wilde, S. A. 1940 Classification of gley soils for the purpose of forest management and reforestation. *Ecology* 21: 34-44.

gen clay or the aluminum clay. The stoppers bore dark stains corresponding to the hydrogen sulfide production. The supernatant liquids gave corresponding suggestions of iron in solution after enough time following the odor test had allowed them to clarify. Opalescence, suggesting colloidal iron, appeared over the natural and potassium clays, and a rusty-colored flocculate was over the gray-colored calcium clay. Thin horizons of gray color, of intensity corresponding to that of the calcium clay, appeared at the top of the natural and potassium clay columns (plate 1). Here the production of soluble iron is suggested, not in an acid soil, but rather at the maximum in the neutral clay saturated with calcium and providing nutritive conditions encouraging microbial performances.

These observations and tests suggest that the process of gleization may center about the presence of calcium in sufficient degrees of saturation of the clay to serve in the bacterial nutrition if the horizon of standing water is to leave its historic record as a bluish gray layer. When such calcium is absent, and apparently because of this calcium deficiency in the bacterial ration, the event of standing water remains unrecorded, regardless of the period of its presence or the possible percolation of the organic matter downward to it.

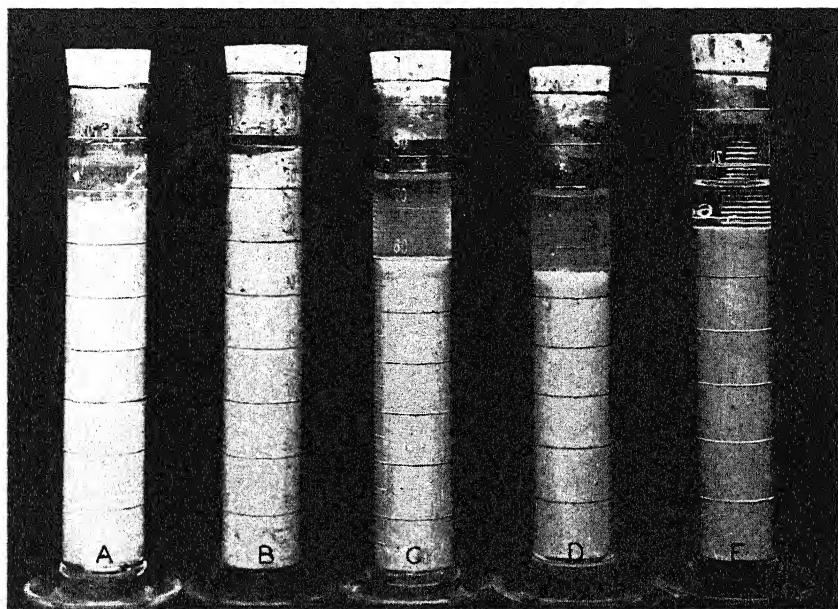
This hypothesis regarding the importance of the degree of calcium saturation in the process of gleization is submitted not as a proved fact but in the hope that students of soils in the field will test it either for verification and acceptance or for disproval and discard.



## PLATE 1

## GLEIZATION OF PUTNAM CLAY

Clays saturated with (A) calcium, (B) aluminum, (C) potassium, (D) no cation, and (E) hydrogen. Gleization, or gray color production, occurred only in the calcium clay.





# A RAPID SOIL TEST—THE 1-GRAM BALL RESISTANCE

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The mechanical properties of the soil are important not only in themselves but also because they give a good idea of many of the physical properties.

TABLE 1

*The 1-gm. ball resistances, sticky points, and ignition losses of variously treated soils*

NATURE OF SOIL	RESISTANCE		STICKY POINT	IGNITION LOSS	RESISTANCE		STICKY POINT	
	Dried at 20°	Dried at 110°			Dried at 20°	Dried at 110°		
	kgm.	kgm.			per cent*	per cent*		kgm.
	Untreated				Na-soil			
Sandy†.....	19.0 ± 0.85	21.0 ± 0.82	25.6	5.2	23.5 ± 0.65	21.2 ± 0.63	27.0	
Terra rossa‡.....	29.7 ± 1.87	28.2 ± 1.11	34.6	9.6	32.7 ± 0.75	6.7 ± 0.85	41.0	
Black sandy†.....	8.0 ± 0.41	9.2 ± 0.25	42.5	7.3	17.0 ± 1.00	18.0 ± 2.38	39.0	
Black clay†.....	31.0 ± 1.29	40.0 ± 0.91	38.6	8.9	29.7 ± 0.48	18.2 ± 1.65	33.5	
Red earth†.....	32.7 ± 0.71	11.2 ± 0.95	32.2	6.1	25.7 ± 0.75	3.2 ± 0.48	34.0	
	Treated with H <sub>2</sub> O <sub>2</sub>				Ca-soil			
Sandy†.....	26.2 ± 1.11	31.0 ± 1	23	4.7	14.5 ± 1.05	21.2 ± 1.18	26	
Terra rossa‡.....	36.7 ± 0.75	28.7 ± 2.10	33	6.7	32.0 ± 0.71	34.5 ± 1.50	36	
Black sandy†.....	11.2 ± 1.11	16.0 ± 0.41	32	5.6	9.5 ± 0.85	12.7 ± 0.63	44	
Black clay†.....	33.5 ± 1.32	38.5 ± 1.56	32	7.3	21.7 ± 1.35	28.0 ± 1.08	37	
Red earth†.....	30.2 ± 1.11	8.7 ± 1.11	34	5.1	28.5 ± 0.87	18.5 ± 0.65	35	

\* Of oven-dry soil.

† Soils poor in organic matter: N content of sandy soil, 0.6 per cent; of red earth, 0.44 per cent.

‡ Soils rich in organic matter: N content of terra rossa, 1.67 per cent; of black sandy soil, 1.22 per cent; and of black clay, 1.44 per cent. The black sandy soil also contains charcoal from burning organic materials.

Note: The errors are calculated according to the formula  $\sqrt{\frac{Sd^2}{n(n-1)}}$  where  $Sd^2$  is the sum of squares of the deviations from the mean and  $n$  is the number of replications. The number of replications was always 4.

Heretofore, however, no rapid test of these mechanical properties has been available. We have, therefore, devised the following method:

To 1 gm. of air-dry soil sufficient water is added to bring the soil to its sticky point. A ball without fissures is then formed and allowed to dry at laboratory temperature and humidity

for about 5 days. Its resistance to crushing is determined by the simple instrument shown in plate 1. Best results are obtained by averaging the figures for four balls.

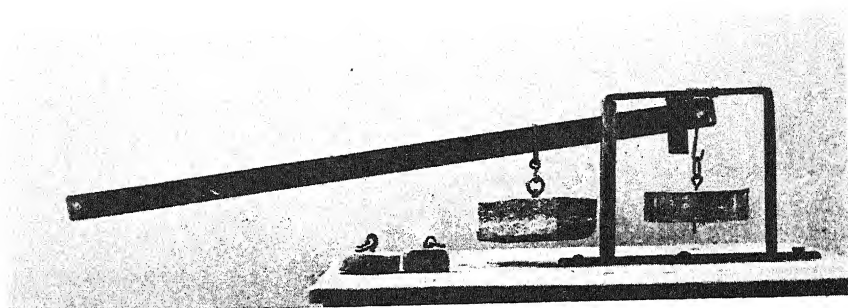
The nature of the absorbed cations influences the resistance of the soil. Sodium and calcium soils were prepared by leaching samples of the same soil with NaCl or CaCl<sub>2</sub> and then with water. As indicated in table 1, soils rich in sesquioxides (red earth and terra rossa) showed greater resistance when saturated with Ca; those poor in sesquioxides (sandy, black sandy, and black clay soils) showed considerably greater resistance when saturated with Na. The soil balls can also be dried in the oven, but such rapid drying greatly influences the results. The resistance of Ca-soils poor in sesquioxides is increased, for example, and that of Na-soils rich in sesquioxides is decreased.

Organic matter decreases the resistance. In general, the soils treated with H<sub>2</sub>O<sub>2</sub> gave the greatest resistance figures; the soils rich in organic matter (terra-rossa, black sandy, and black clay soils) gave small resistance figures in comparison with their sticky points.

The results recorded in this paper suggest that the 1-gm. ball resistance affords a possible clue to other important properties of the soil. It should be interesting, for example, to study the relation between this resistance and the physical and chemical composition of the clay fraction, organic matter, etc. Such work, however, can be done only in well-equipped laboratories having at their disposal a great number of soil samples from different countries.

#### PLATE 1

INSTRUMENT FOR DETERMINING RESISTANCE TO CRUSHING OF A 1-GM. BALL OF SOIL





# THE BASE-EXCHANGE CAPACITY OF THE ORGANIC AND INORGANIC FRACTIONS OF SEVERAL PODZOLIC SOIL PROFILES<sup>1</sup>

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It is well known that both the organic and the inorganic materials of a soil play an important part in the base-exchange reaction. Most of the research work on base exchange in soils, however, has dealt with the reaction in the soil as a whole, or with just the mineral portion, and very few investigations have been concerned with the relative importance of the organic and inorganic fractions in this phenomenon. Only in recent years have investigators directed their attention to the base-exchange capacity of the organic matter of the soil; consequently, the exchange reaction of this fraction is as yet little understood.

Baver (2) found that 30 to 60 per cent of the total base-exchange capacity of surface soils could be attributed to the organic portion; Mitchell (3) concluded that the exchange capacity of the organic portion of surface soils constitutes 41 to 65 per cent of the total; and Olson and Bray (4) found that the organic base-exchange capacity constituted 6.8 to 43.4 per cent of the total base-exchange capacity of the surface soils studied. These figures tend to show the importance of the organic exchange capacity and suggest that heretofore not enough importance has been attached to this fraction.

Data on the base-exchange capacity of soil horizons is fairly meager, and almost no attention has been directed to the part played in this reaction by the organic content of different soil horizons. It is the purpose of this paper to report the base-exchange capacity of the organic as well as the inorganic fractions in the A<sub>1</sub>, A<sub>2</sub>, B<sub>1</sub>, B<sub>2</sub>, and C horizons of six podzolic profiles and to study their individual effects on the total base-exchange capacity.

## DESCRIPTION OF THE SOILS STUDIED<sup>3</sup>

This investigation was confined to six podzolic soil types of the lower peninsula of Michigan, a description of each of which follows. All samples were from virgin areas and were collected by horizons.

<sup>1</sup> Contribution from the soils section of the Michigan Agricultural Experiment Station. Authorized by the director for publication as journal article No. 458 n.s.

<sup>2</sup> Graduate student and assistant professor respectively.

<sup>3</sup> Acknowledgement is made to A. H. Mick for his descriptions of the soil types.



*Isabella loam*

Isabella loam is a well-drained soil developed from calcareous, morainic drift under a hardwood forest in which beech, maple, hemlock, ash, and basswood were the dominant species together with a few scattered white pine. The forest floor is covered with a 2- to 4-inch litter of decaying leaves, which overlie a relatively homogeneous layer of black, granular, neutral to slightly alkaline organic material ranging from  $\frac{1}{4}$  to 1 inch in thickness. Underlying this is a 2- to 6-inch layer of harsh, platy, ash-gray loamy sand to sandy loam, which sometimes is acid in reaction and somewhat stained at the top by infiltrating organic matter. Below the podzolized layer, 10 to 14 inches of transitional sandy loam or loam grade into reddish brown, acid, highly structured sandy clay. This layer is characterized by a nut or blocklike structure, the surfaces of the blocks being covered with a dark brown coating and many minute roots. In the lower part free carbonates are not infrequently observed. Massive, calcareous, pinkish or reddish brown till clay is encountered at depths ranging between 2 and 6 feet below the surface.

*Selkirk loam*

Selkirk loam is a well-drained heavy-textured soil which developed on the clayey calcareous lake plains under a mixed forest of pine and hardwoods. From the surface downward this soil type consists of a dark colored humus layer 1 to 3 inches thick; 4 to 8 inches of ash-gray fine sandy or silty loam; pale yellowish brown, in many places slightly mottled, sandy loam to clay loam 3 to 8 inches thick; and finally pale reddish brown impervious clay. The first three layers may be acid, but the heavy subsoil is alkaline and the substratum contains a high percentage of carbonates.

*Rubicon sand*

Rubicon sand is a well-drained, pervious soil of the dry pine plains. Under a virgin cover of red and white pines, 1 to 3 inches of litter accumulate. A one-fourth-inch humus layer is underlain by the characteristic ash-gray podzolized sand, which ranges between 2 and 8 inches in thickness. This layer in turn overlies and grades into pale yellowish brown loamy sand 4 to 6 inches thick, slightly indurated in places. The substrate consists of pale yellow, loose, pervious sand, which extends more than 7 feet below the surface.

*Ogemaw sandy loam*

Ogemaw sandy loam is a ground-water podzol of the poorly drained pine plains. The surface is characteristically rather mucky under a relatively deep accumulation of litter; this soggy humus layer, 2 or 3 inches thick, overlies 4 to 8 inches of conspicuously white sand or loamy sand. Directly below the leached layer is a dark coffee-brown heavily indurated, sandy hardpan, which in places may be as thick as 12 inches. Through a thin transition layer the brown color rapidly changes to the drab, dingy gray of waterlogged sand.

At a depth varying between 3 and 5 feet, heavy impervious lacustrine clay is encountered. The subsoil contains a small percentage of carbonates.

#### *Kalkaska loamy sand*

Kalkaska loamy sand has developed under a hardwood forest of beech, maple, and hemlock on the dry sand plains. The surface litter decomposes rapidly to produce a thin, dark brown, neutral, humus layer which overlies 2 to 4 inches of dark gray loamy sand. This layer grades downward into 2 to 5 inches of ash-gray loamy sand which may be acid in reaction. Underlying this podzolized horizon are 4 to 10 inches of dark coffee-brown loamy sand which in many places is slightly indurated. This brown color rapidly fades, and at a depth of 18 to 24 inches below the surface the pale yellow, pervious, sandy substratum is encountered.

#### *Emmet loamy sand*

Emmet loamy sand is a light-textured soil which has developed beneath a hardwood cover in the alkaline, sandy morainic drift. Under a moderate accumulation of litter and a thin, grayish brown, neutral to slightly acid humus layer, are 2 to 3 inches of dark gray stained loamy sand to sandy loam, which grades into the harsh, platy, compact, ash-gray leached horizon. This podzol horizon is acid in reaction and ranges between 2 and 6 inches in thickness. It is underlain by 6 to 10 inches of brownish yellow, acid, loamy sand, which in turn grades downward into the sandy and gravelly parent drift material.

#### EXPERIMENTAL METHODS

The air-dried samples were screened and ground to pass a sieve with openings 0.84 mm. in diameter. The percentage of carbon was determined by dry combustion, and the organic matter content was then calculated by using the conventional factor 1.72. A correction for the carbonate content of several samples was made.

The exchange capacities were determined by placing the equivalent of 25 gm. of the oven-dry samples in the apparatus described by Russel (5). The samples were then leached with neutral normal ammonium acetate, the ammonia was removed by neutral normal calcium acetate, and the milli-equivalents of ammonia were determined in the leachate.

Exchangeable calcium and magnesium were determined in the ammonium acetate leachate by precipitation as calcium oxalate and as magnesium ammonium phosphate.

Since Alexander and Byers (1) concluded that hydrogen peroxide could not be used to determine the total amount of organic matter present in the soil, the organic fraction in this investigation was destroyed by ignition, after the method of Mitchell (3), who found that ignition at 350° to 400°C. for 7 or 8 hours produced a well-oxidized sample but did not destroy or change the base-

exchange capacity of the inorganic material. A sample equivalent to 25 gm. of oven-dry soil was ignited at 400°C. for 7 hours. It was then cooled, placed in the percolation tube, and the exchange capacity was determined as before.

The difference between the base-exchange capacity of the original sample and that of the sample in which the organic matter had been destroyed represents the base-exchange capacity of the organic matter in the oven-dry soil. The latter exchange capacity was then calculated to milliequivalents per 100 gm. of organic matter by the following formula:

$$\frac{\text{Organic exchange capacity expressed in} \\ \text{millequivalents per 100 gm. of soil} \times 100}{\text{Percentage organic matter present in the soil sample}} = \frac{\text{Absolute exchange capacity} \\ \text{of the organic matter}}$$

#### DISCUSSION OF RESULTS

Figure 1 shows graphically the variation between the base-exchange capacities of the different horizons. As a well-developed B horizon usually consists of an accumulation of materials, one would naturally expect this horizon to possess a fairly high base-exchange capacity, relative to the horizons immediately above and below it. The graphs in figure 1, with one exception, show a very definite inflection in the total base-exchange capacity curves, and this increase in the exchange capacity corresponds exactly with the horizons sampled in the field as horizons of accumulation.

From the total base-exchange capacity curves one must conclude that all the soils studied, with the exception of Rubicon sand, possess more or less well-defined B horizons. Furthermore, slight differences in the B horizons are indicated by these curves. Among the soils studied, perhaps the best illustration of a well-developed horizon of accumulation is shown by Isabella loam, and this is reflected in the exchange capacity curve by a sharp inflection. The corresponding curve for Selkirk loam has a slightly different shape, showing a much less prominent inflection and a somewhat lower exchange capacity. The horizon of accumulation is less well defined.

The total exchange capacity curves for Emmet and Kalkaska loamy sands clearly indicate the presence of a B horizon developed in a sandy profile. The inflection in the exchange capacity curve for the former soil type is a little more abrupt than that for the latter, and would seem to indicate the development of a fairly thin B horizon, whereas this horizon in the Kalkaska soil type is possibly slightly thicker and grades less abruptly into the underlying sandy horizon.

A distinct difference in the shape of the curve for the Ogemaw soil type is noted. Curiously, the inflection in this curve is not particularly abrupt and the exchange capacity increases slightly in passing from the B to the C horizon. This probably indicates a fairly fine textured C horizon, relative to the B, and a thin transition zone between the B and C horizons.

The curve for Rubicon sand indicates that it possesses the least well-defined

horizon of accumulation of all the soils studied. The  $B_1$  horizon shows evidence of some accumulation, since the base-exchange capacity is greater than that of the  $B_2$  and C horizons. A slight accumulation of organic matter in the B horizon is indicated by curve 3.

The base-exchange capacity curves for the organic and inorganic fractions in the various horizons bring out some striking differences. The curve for the

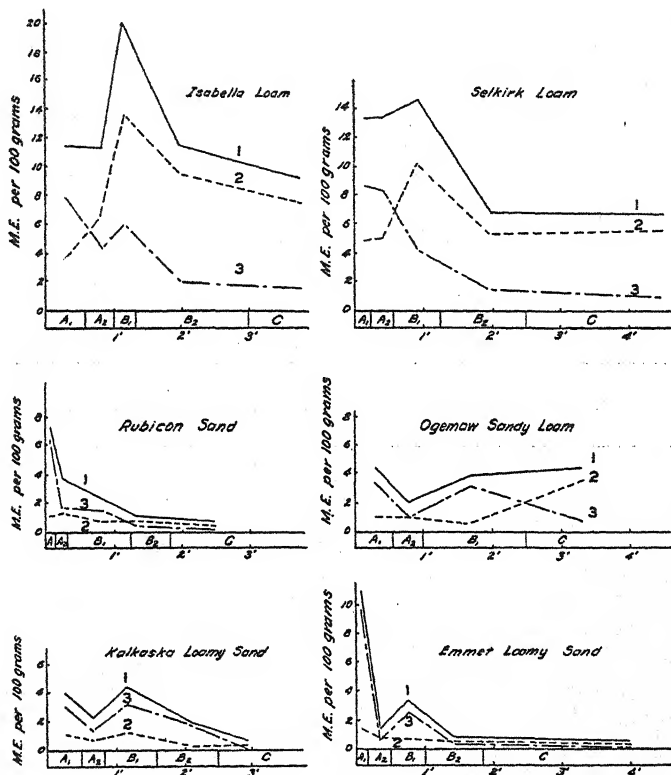


FIG. 1. BASE-EXCHANGE CAPACITY OF THE DIFFERENT HORIZONS IN SIX PODZOLIC SOIL PROFILES

1. Total base-exchange capacity. 2. Base-exchange capacity of the inorganic portion of the soil. 3. Base-exchange capacity of the organic fraction of the soil.

organic portion in Isabella loam, curve 3, shows a definite increase in the  $B_1$  horizon, and this corresponds to a similar increase in the organic matter content of this horizon (table 1). Curve 3 for Selkirk loam shows no inflection, and this in turn corresponds with the progressive decrease of organic matter content with increasing depth in the profile (table 1). The curves for the inorganic fractions of these two soils (curve 2) are very similar, and they illustrate the fact that mineral material has accumulated in the B horizons and that most of

the base-exchange capacity of the B<sub>1</sub>, B<sub>2</sub>, and C horizons in these soil types is due to the inorganic portion.

Isabella and Selkirk loams have high base-exchange capacities in relation to the other soils, because they are finer textured and possess a greater specific surface. The effect of the organic matter is therefore less pronounced than in the other four soil types, and it follows that the mineral fraction would be the dominant one in the base-exchange reaction of the Isabella and Selkirk samples.

The exchange capacity curves for the organic and inorganic fractions of the Emmet, Kalkaska, and Ogemaw soils are very similar (fig. 1). The curves for the organic portion show very definite inflections in the B horizons; these inflections correspond to accumulations of organic matter (table 1). The total exchange capacity is but little influenced by the inorganic portion; virtually all of it can be attributed to the organic matter present. A slight accumulation of inorganic material in the B horizon is shown in the Kalkaska soil.

TABLE 1  
*Organic matter content of the soil horizons*

SOIL TYPE	ORGANIC MATTER IN HORIZON				
	A <sub>1</sub>	A <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>	C
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Isabella loam.....	3.38	0.76	1.16	0.25	0.31
Selkirk loam.....	3.80	3.11	0.87	0.65	0.49
Rubicon sand.....	2.50	1.21	0.91	0.49	0.16
Ogemaw sandy loam.....	1.70	0.43	1.27	—	0.55
Kalkaska loamy sand.....	1.67	0.57	1.60	0.79	0.12
Emmet loamy sand.....	5.37	0.64	1.62	0.48	0.14

The peculiarities of the Ogemaw sample, previously pointed out, can be more easily explained by a closer examination of curves 2 and 3, figure 1. The curve for the organic exchange capacity shows a very definite inflection in the B horizon, which is associated with an accumulation of organic matter (table 1). The exchange capacity of the inorganic portion becomes progressively greater with increasing depth below the B horizon, and this accounts for the gradual rise of the total base-exchange capacity curve. If one more sample had been available (B<sub>2</sub>), curve 1 would undoubtedly have shown more of an inflection, for at this point the influence of the inorganic portion would not have been the dominant factor. Evidently in the lower B<sub>1</sub> or upper C horizon the proportion of fine-textured materials has increased, or the nature of this fraction is different from that in the other sandy types, possibly because of different environmental conditions.

In each of the six graphs of figure 1, the percentage of organic matter (table 1) follows the number 3 curves very closely; in other words, the base-exchange capacity of the organic fraction usually increased as the percentage of organic matter increased. The coefficient of correlation was found to be +.90, which

shows that there is a very close relationship between the organic base-exchange capacity and the percentage of organic matter.

The percentage of the total base-exchange capacity due to the organic and the inorganic fractions of the various horizons has been plotted in figure 2. The dominant role played by the organic matter in the base-exchange reaction of the sandy types is readily noted. Similarly, it can be seen that the organic fraction of the  $A_1$ , and in several cases the  $A_2$  horizons, possesses a much greater base-exchange capacity than does the inorganic portion of these same horizons.

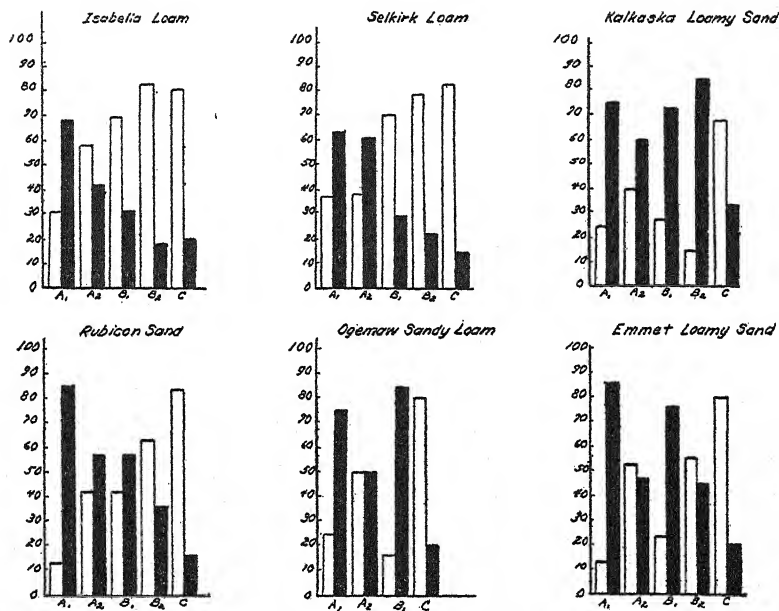


FIG. 2. PERCENTAGE OF THE TOTAL BASE-EXCHANGE CAPACITY DUE TO THE ORGANIC AND INORGANIC FRACTIONS OF THE VARIOUS SOIL HORIZONS

Solid bars, organic; hollow bars, inorganic

The Isabella and Emmet soils are the only two in which the inorganic fraction of the  $A_2$  horizon showed a greater exchange capacity than did the organic portion. With increasing depth in these two profiles, with the exception of the  $B_1$  horizon of the Emmet and the C of the Isabella soil, one notes a progressive decrease in the percentage of the total base-exchange capacity due to organic matter and an increase in the percentage due to the inorganic fraction. Except for the  $B_1$  horizons of both soils and the C horizon of the Isabella, this is associated with the progressive decrease of organic matter content with increasing depth. One other soil type, Ogemaw sandy loam, is noted in which the organic fraction of the  $A_2$  horizon is not the dominant fraction. Here the influences of the organic and inorganic portions on the exchange capacity are equal. In the B horizon 84 per cent of the base-ex-

change capacity is due to organic matter, showing considerable accumulation of this material.

The diagram for Kalkaska loamy sand (fig. 2) presents a rather striking picture in that the organic matter accounts for over 60 per cent of the base-exchange capacity in all horizons within the solum. In the B horizon as much as 85 per cent of the base-exchange capacity is due to organic matter, which indicates considerable accumulation of this fraction.

The range in percentage of the total base-exchange capacity due to the organic matter in all the horizons studied is tabulated as follows by way of summary:

HORIZON	MAXIMA AND MINIMA PERCENTAGES OF TOTAL BASE-EXCHANGE CAPACITY DUE TO ORGANIC MATTER
A <sub>1</sub>	63.9-86.2
A <sub>2</sub>	41.7-61.9
B <sub>1</sub>	29.4-84.2
B <sub>2</sub>	17.4-85.0
C	16.2-33.3

The absolute exchange capacity of the soil organic matter (milliequivalents per 100 gm. of organic matter) is plotted in figure 3. It is apparent that there is a wide variation in the absolute exchange capacity of the organic fraction, not only in the different soil profiles but also between horizons within the same profile. This value for the organic matter in Selkirk and Isabella loams is very much greater than that for any of the other soil types studied. This dissimilarity undoubtedly reflects a difference in the chemical nature of the organic matter present. It is logical to assume that the soil organic matter developed in Emmet loamy sand, for example, has undergone a slightly different type of degradation from that formed in Selkirk loam, because of differences in pH, microorganisms, drainage, calcium content, and other factors. For the same reasons one would expect the organic matter in the various soil horizons to consist of material differing slightly in chemical composition. During the simple process of eluviation certain of the more soluble fractions of the organic matter, and groups or portions of the organic material easily hydrolyzed or split off, will be transported to lower horizons in the greatest quantities, thereby affording a partial separation of the various organic fractions.

The absolute exchange capacity of the organic fraction in the A<sub>2</sub> horizon of Isabella loam is extremely high and might be due to the very low organic matter content, 0.76 per cent, of this horizon. This necessitates multiplying the results by a factor greater than 100, and thus a slight error in determining either the exchange capacity or the percentage organic matter would result in a large final error.

The exchangeable calcium and magnesium found in the various horizons of the Rubicon, Kalkaska, and Emmet soil samples are plotted in figure 4.

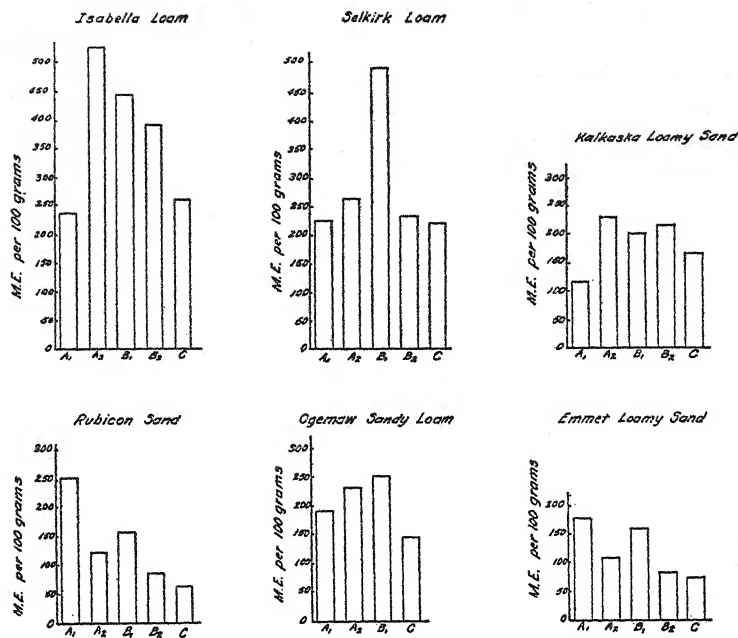


FIG. 3. ABSOLUTE EXCHANGE CAPACITY OF THE ORGANIC FRACTION PRESENT IN THE VARIOUS SOIL HORIZONS, EXPRESSED IN MILLIEQUIVALENTS PER 100 GM. OF ORGANIC MATTER

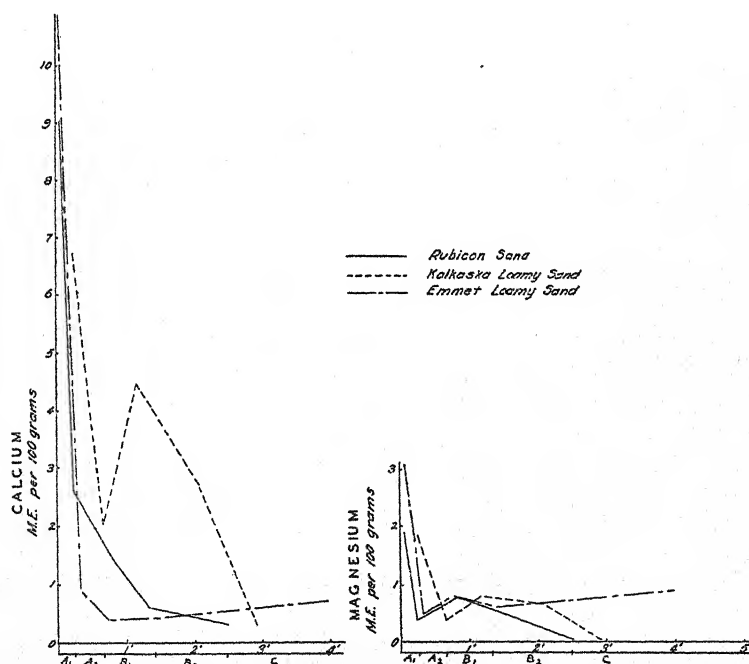


FIG. 4. MILLIEQUIVALENTS OF EXCHANGEABLE CALCIUM AND MAGNESIUM FOUND IN VARIOUS HORIZONS OF THREE PODZOLIC SOILS



Exchangeable calcium and magnesium were not determined in the other samples because most of their horizons contained free carbonates.

The surface horizons of all three soils contained a relatively large amount of calcium, which was probably retained, primarily, by the organic matter present. In the  $A_2$  horizons, however, the amount of exchangeable calcium decreased tremendously and, with the exception of the B horizon in the Kalkaska sample, continued to decrease rapidly with increasing depth. The Emmet sample below a depth of 1 foot, however, showed a gradual increase in exchangeable calcium. Kalkaska loamy sand shows an accumulation of calcium in the B horizon, and this may account, in part, for the pronounced effect organic matter had on the base-exchange capacity of this sample (fig. 1). Although calcium would tend to stabilize the organic matter and prevent its loss by leaching, the percentage of organic matter present in the  $B_2$  horizon of the Kalkaska sample is only slightly greater than that in the corresponding horizon of the Emmet soil. Calcium, however, may have influenced the chemical nature of the organic matter in this horizon, since its absolute exchange capacity is much greater than that of the organic matter in the  $B_2$  horizon of the Emmet sample (fig. 3). Thus, indirectly the presence of exchangeable calcium probably increased the base-exchange capacity of the Kalkaska  $B_2$  horizon.

All three of the soils show a slight accumulation of magnesium in their respective B horizons.

#### SUMMARY AND CONCLUSIONS

The base-exchange capacity of each of several horizons of six podzolic soils—Isabella and Selkirk loams, Rubicon sand, Ogemaw sandy loam, and Kalkaska and Emmet loamy sands—was studied. The base-exchange capacities of the organic and inorganic fractions of these same samples were also investigated.

A considerable variation in the total base-exchange capacity was noted among the soils studied. Similarly, a wide variation in the base-exchange capacity of horizons of the same profile was shown.

The base-exchange capacity of Isabella and Selkirk loams was due primarily to the inorganic fraction of the soil, whereas the base-exchange capacity of the four sandy soil types was due principally to the organic fraction.

The organic matter present in the  $A_1$  horizons studied accounted for 64 to 86 per cent of the base-exchange capacity of these horizons. Similarly, the organic base-exchange capacity constituted 42 to 62 per cent, 29 to 84 per cent, and 17 to 85 per cent of the total base-exchange capacity of the  $A_2$ ,  $B_1$ , and  $B_2$  horizons, respectively.

The coefficient of correlation between the organic exchange capacity and the percentage organic matter was  $+0.90$ .

A wide variation in the absolute exchange capacity of the organic fraction was noted, not only among the different soil profiles but also between hori-

zons within the same profile. It was concluded that this variation was caused by differences in the chemical nature of the organic matter.

Exchangeable calcium and magnesium were determined in 15 samples. The surface horizons were relatively high in calcium, which was probably retained, primarily, by the organic matter. The Kalkaska sample showed an accumulation of exchangeable calcium in the B horizon, and it was suggested that, indirectly, this might account, in part, for the pronounced effect organic matter had on the base-exchange capacity of this sample.

All three of the sandy soil types showed a slight accumulation of exchangeable magnesium in the B horizons.

In the soils studied, a relation between the increased base-exchange capacity of the B horizons and the extent of development of these horizons was proposed.

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ORGANIC PHOSPHORUS IN SOILS: II. THE NATURE OF THE  
ORGANIC PHOSPHORUS COMPOUNDS. A. NUCLEIC ACID  
DERIVATIVES. B. PHYTIN<sup>1</sup>

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A. NUCLEIC ACID DERIVATIVES

Nucleic acid derivatives have been shown to be present in preparations from soil containing considerable concentrations of organic phosphorus. The exact nature of the compounds present and of their relation to the organic phosphorus, however, remains obscure (30). Further evidence on this subject was sought by studying material obtained from the A<sub>1</sub> horizon of a podzol soil.

*Experimental*

The material, obtained as described in a previous paper (8), was somewhat purified by thrice repeated solution in water containing a little ammonia and by reprecipitation in acidified 80 per cent alcohol. The products, P 12 from experiment G, and P 13 from experiment H, were then analyzed for phosphorus and for nitrogen by the micro-Kjeldahl method of Pregl (23). These analyses are recorded in table 1.

The nitrogen contents of the preparations were very low and apparently were unrelated to the phosphorus contents. The N/P ratios are only 0.63 and 0.33, whereas that for ribonucleic acid is 1.69, and hence the N content is much lower than it would be if the organic phosphorus consisted only of simple nucleotides. This indicates the presence of nonnitrogenous phosphorus compounds, such as phosphoric esters of inositol.

Purine nitrogen was determined by the method of Levene<sup>1</sup> and co-workers (15, 28). Samples weighing about 200 mgm. were refluxed for 4 hours with 10 cc. of 5 per cent H<sub>2</sub>SO<sub>4</sub>. The free purine was separated by precipitation with Ag<sub>2</sub>SO<sub>4</sub>, and N was determined after decomposition of the Ag salt by HCl. A determination on ribonucleic acid (Eastman) showed 66.0 per cent of the total N as purine N, which is approximately the theoretical amount.

<sup>1</sup> Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Quebec, Canada. Macdonald College Journal Series No. 149. Taken in part from a thesis by W. J. Dyer presented in partial fulfillment of the requirements for the degree of doctor of philosophy.

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The nucleotide preparations, however, yielded only small amounts of purine N, from P 12, 7.4 per cent, and from P 13, 11.4 per cent of the total N present.

When attempts were made to identify the purine constituents obtained in the same way from about 5 gm. of the combined preparations, following the methods of Levene and his co-workers (15, 16, 25), no adenine or guanine could be detected. Instead, when the HCl solution obtained by decomposing the Ag-purine precipitate was treated with NaOH, a precipitate appeared which then redissolved on further addition of NaOH, neutralizing the solution to congo red.

The gel-like precipitate was insoluble in dilute HCl or  $\text{H}_2\text{SO}_4$  and soluble in  $\text{NH}_4\text{OH}$  or NaOH. Guanine and hypoxanthine are insoluble in  $\text{NH}_4\text{OH}$  and soluble in dilute HCl, and adenine is soluble in both. Xanthine, on the other hand, forms salts with strong acids which are readily hydrolyzed on dilution or partial neutralization with the precipitation of the free substance, and is readily soluble in alkalis. The murexide test on the purine solution was positive. It is given by xanthine and guanine. Weidel's test, not given by guanine, was also positive, confirming the presence of xanthine. The

TABLE 1  
*Analyses of reprecipitated "nucleotide" preparations*

SAMPLE	P	N	N/P
	<i>per cent</i>	<i>per cent</i>	
P 12	4.50	2.84	0.63
P 13	5.88	1.92	0.33

quantity obtained was too small, however, to enable its isolation in a pure state. Inorganic phosphate and pentose sugar, the other constituents of xanthylic acid, were also present in the hydrolyzate. The sugar did not respond to the Feulgen and pine shaving tests; hence it was not a deoxy sugar, and may be presumed to have been ribose.

The filtrate from the purines released by dilute acid hydrolysis was freed from Ag with  $\text{H}_2\text{S}$  and subjected to drastic hydrolysis with 25 per cent  $\text{H}_2\text{SO}_4$  at  $175^\circ\text{C}$ ., with the intention of releasing the pyrimidine bases. Sulfuric and phosphoric acids were removed by means of  $\text{Ba}(\text{OH})_2$ , and excess Ba was precipitated by  $\text{H}_2\text{SO}_4$ . When  $\text{Ag}_2\text{SO}_4$  was added to the slightly acid solution a copious precipitate of Ag-purine separated. This was removed, and when the filtrate was made alkaline with  $\text{Ba}(\text{OH})_2$ , a small precipitate formed corresponding to Ag-pyrimidine.

The Ag-purine precipitate was decomposed with HCl, and the filtrate from AgCl was gradually neutralized. No material separated until the congo red end point was reached, but when neutrality was approached, a dense, flocculent precipitate of impure guanine formed. This was removed and dissolved in

hot dilute  $\text{H}_2\text{SO}_4$ . The solution was decolorized with charcoal, and the guanine was again precipitated by the addition of  $\text{NH}_4\text{OH}$  in excess, when it appeared as nearly white, granular material. This product readily formed a picrate, and gave a strong murexide reaction, confirming its identity as guanine. It was further purified according to the directions of Levene and Bass (16, p. 110), and a final yield of about 200 mgm. of almost pure guanine was obtained.

The supposedly Ag-pyrimidine precipitate was decomposed with  $\text{H}_2\text{S}$ , and the filtrate from  $\text{Ag}_2\text{S}$  was decolorized with charcoal and concentrated. No material separated, and no crystals formed on the addition of picric acid. The Wheeler and Johnson test was negative. This behavior showed the absence of any appreciable amount of pyrimidine substances.

### *Discussion*

Preparations rich in organic phosphorus obtained from soil have been shown repeatedly to release nucleic acid constituents on hydrolysis. Pentose sugar and phosphoric acid have been observed in every instance, though the basic constituents varied in the different preparations.

Shorey (27) obtained hypoxanthine, adenine, and cytosine from his various preparations, although not all of these were necessarily present in any one of them.

Schreiner and Lathrop (26) separated such materials from two soils and from the same soils after treatment with live steam in an autoclave. The yields were much smaller from the steam-heated soils, indicating that hydrolysis had taken place. Furthermore, whereas without heating, only xanthine from the first soil and hypoxanthine from the second were found free in the soil, after heating, hypoxanthine, adenine, and cytosine were also obtained from the first soil, and xanthine, guanine, and cytosine were also obtained from the second. Shorey and Schreiner and Lathrop had no means at their disposal for the detection of uracil; hence their results do not exclude the possibility that uracil was present either in the preparations or free in the soils.

Bottomley (5) prepared similar material from samples of English peat soils by a modified method, using  $\text{NaHCO}_3$  as an extractant. He observed that mild acid hydrolysis failed to release more than a trace of purine substance. On high-temperature hydrolysis with 25 per cent sulfuric acid, however, considerable quantities of adenine were obtained. The pyrimidine uracil was also released by this hydrolysis. Bottomley concluded that the original substance was an adenine-uracil dinucleotide derived from a partial decomposition of plant nucleic acid. The other basic constituents of ribonucleic acid, guanine and cytosine, were found in the alcoholic filtrate from the separation of the product, indicating that they were present in the soils in a free state.

In this laboratory Wrenshall and McKibbin (29) prepared material from muck and mineral soils, from which, after drastic hydrolysis, they also ob-

tained adenine and uracil. The sample hydrolyzed was a composite from several soils, but material from the muck soil predominated, which may account for the coincidence of this result with that of Bottomley. Since the existence of an adenine-uracil dinucleotide appeared to have been refuted, it was concluded that the product was an impure mixture of mononucleotides.

In the present study of material obtained from the  $A_1$  horizon of a podzol, xanthine was detected after mild hydrolysis, and guanine was obtained after drastic hydrolysis. This is the first time that the presence of these two substances has been shown directly, although Schreiner and Lathrop obtained good evidence for their presence in such material. No pyrimidines were detected, but the amount of guanine obtained was sufficient to account for most of the nitrogen in the material.

The foregoing evidence, considered by itself, seems to afford conclusive proof of the widespread occurrence in soil of "nucleic acids," using that term in its broadest sense. The presence in the preparations of phytin and perhaps other phosphates of inositol, as demonstrated in the following pages and by the recent work of Yoshida (31), however, introduces a new factor which compromises that proof somewhat. It would be possible to explain the results which have been quoted on the basis of mixtures of inositol phosphates and nucleosides. Without prejudice, therefore, to the still strong probability that certain nucleic acids are usually present, it would seem prudent to reserve conclusions in this regard until final proof is forthcoming.

In commenting on the accumulated evidence, it is to be noted that all the bases of ribonucleic acid and their deamination products have been obtained at one time or another from these materials, although no instance has been recorded where they were all present together. This indicates that we are dealing with degradation products of ribonucleic acid. In at least one important respect, however, the soil compounds are different from known nucleic acid derivatives, that is, in the hydrolytic release of the purine constituents. The purines of ribonucleic acid and of the individual ribonucleotides are released by mild acid hydrolysis. In the present work the larger part of the purine was not released by mild hydrolysis, and guanine was obtained only after drastic treatment such as is required for the release of pyrimidines. Bottomley recorded similar results in connection with the release of adenine, as has been noted. A close reading of Shorey's work with this observation in mind suggests that he, too, found it necessary to employ 25 per cent  $H_2SO_4$  in order to release the purines. This result is taken to indicate that the purines in the soil preparations are not linked to ribose in the same manner as are the purines in the known ribonucleotides or nucleosides. It is possible that this difference in structure is associated with the resistance of the soil compounds to enzymatic hydrolysis. In this connection it should be pointed out that there are still important problems to be solved with regard to the structure of nucleic acids and to their partial resistance to enzymatic hydrolysis (11).

### Summary

Analysis of soil "nucleotide" preparations showed that their N/P ratios were very low, indicating the presence of nonnitrogenous compounds such as phytin.

Dilute acid hydrolysis released only small amounts of purine, which was identified as xanthine. A pentose, presumably ribose, and phosphoric acid were also released by this hydrolysis.

Guanine was isolated after drastic acid hydrolysis. The amount obtained accounted for most of the nitrogen of the preparation. No other purine or pyrimidine substances were detected.

In discussing the evidence relating to nucleic acid derivatives in soil, it is pointed out that these substances have not been proved to account for an appreciable part of the organic phosphorus, although it is highly probable that they do. A difference between the soil compounds and ordinary nucleic acid derivatives is seen in the difficulty of releasing purines from the former by acid hydrolysis.

### B. PHYTIN

Evidence already cited had shown the probability that phytin is present in soil and in the organic phosphorus preparations obtained from soil. Direct attempts were therefore made to isolate this compound.

### Experimental

*Isolation and properties of soil phytin.* A sample of soil organic phosphorus preparation containing 4.32 per cent P was dissolved in dilute HCl and titrated with  $\text{FeCl}_3$  solution according to the method of Rather (24). The presence of a considerable amount of phytin was indicated, but the end point was so uncertain that the result was considered only qualitative. The method of McCance and Widdowson (18) was then applied. An excess of  $\text{FeCl}_3$  solution was added to a  $N/6$  HCl solution of the sample, and the dark-brown precipitate which formed was coagulated by heating. This was separated and then decomposed with 1 per cent NaOH by heating for 15 minutes in a boiling-water bath. The  $\text{Fe}(\text{OH})_3$  was removed, and the organic phosphorus in the solution was determined. The results showed 2.12 per cent phytin phosphorus, which was 49 per cent of the total phosphorus of the preparation.

The material precipitated by  $\text{FeCl}_3$  was obviously very impure. Only a small amount was prepared, because of the scarcity of the original soil preparation. An attempt to isolate inositol from this product was unsuccessful.

A means was then sought by which phytin could be isolated directly from soil. As phytin was known to be very resistant to alkaline hydrolysis, its stability toward oxidation by alkaline hypobromite was investigated to determine whether this treatment could be used to destroy extraneous organic matter in soil extracts.



A quantity of authentic phytin was prepared from wheat bran by the method of Boutwell (6). This product contained 21.33 per cent P on the oven-dry basis, as compared with 20.96 for  $C_6H_6O_{24}P_6Ca_6$ . To 5-cc. aliquots of a 0.5 per cent solution of phytin in 0.1 *N* HCl were added 5 cc. of 5 *N* NaOH, 5 cc. of saturated bromine water, and 10 cc. of water. After boiling for various periods, the solutions were acidified, and the excess  $Br_2$  was boiled off. The results of phosphate determinations on these solutions are recorded in table 2.

Only a slight degree of decomposition was observed in these tests, and this may have been due to acid and alkaline hydrolysis as well as to the action of hypobromite. Since  $\alpha$ -humus is very readily oxidized by NaOBr or NaOCl (7, 9, 19), it seemed practical to use such a treatment as a preliminary to the direct separation of phytin from soil extracts.

A 50-gm. sample of the Halliday soil, No. 7, (8) was leached with *N* HCl, and treated with 500 cc. of 2 per cent NaOH solution on the steam bath. The

TABLE 2  
*Decomposition of phytin by alkaline bromine*

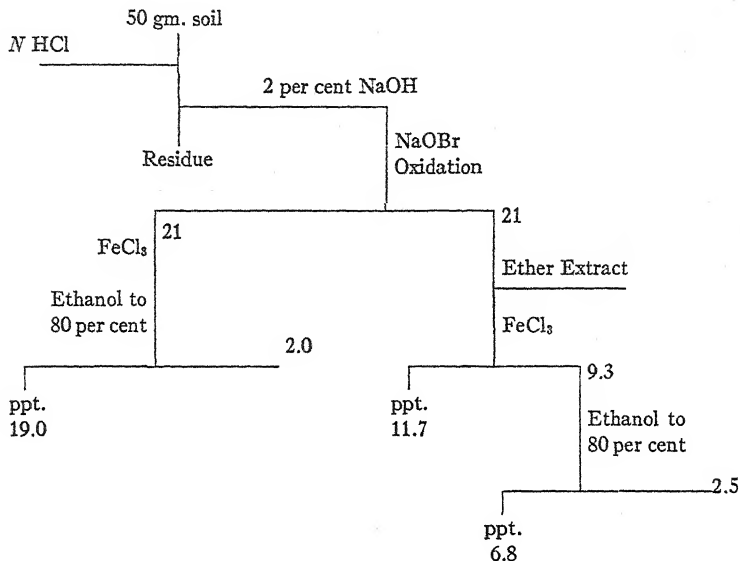
TEST NUMBER	TIME OF BOILING	PHYTIN P PRESENT	INORGANIC P FOUND	DECOMPOSITION	
	minutes	mgm.	mgm.	mgm. P	per cent
1	0	4.0	0.032	.....	...
2	30	4.0	0.139	0.107	2.7
3*	40	4.0	0.300	0.268	6.7
4	60	4.0	0.194	0.162	4.0
5†	30	4.0	0.143	0.111	2.8

\* Boiled almost dry after acidification.

† Excess Br was added in this test.

liquid was separated, brought to boiling, and treated with a solution containing  $Br_2$  and NaOH in equivalent amounts until most of the dark color was discharged. After acidification with HCl, the excess  $Br_2$  was boiled from the extract. A solution of ferric chloride was added to half of the extract, which was then digested for 20 minutes on the steam bath. No precipitation occurred. Feustel and Byers (9) observed that a large proportion of the bromination products of  $\alpha$ -humus are ether-soluble and that oxalic acid is one of the products. It was thought that these compounds might be preventing the precipitation of ferric phytate. The remaining half of the solution was extracted four times with 50-cc. portions of ether. On the addition of  $FeCl_3$ , a precipitate formed immediately and was removed. Further precipitation occurred on the addition of four volumes of ethanol. The organic phosphorus in the nonether-extracted portion was almost completely precipitated when enough ethanol was added to make the final concentration of ethanol 80 per cent. The results of this experiment are summarized in the following diagram,

where figures are given to show milligrams of organic phosphorus in the various fractions:



This experiment showed the practicability of the Br<sub>2</sub> treatment, although the products appeared to be very impure. A larger amount of the Halliday soil was now extracted. A 500-gm. sample was leached with 1000 cc. of *N* HCl and heated overnight on the steam bath with 5 liters of 2 per cent NaOH. The extract was brought to boiling and treated with Br<sub>2</sub> dissolved in NaOH until nearly decolorized, when it was acidified with HCl and the excess Br<sub>2</sub> boiled off. The solution then contained 172 mgm. of organic P. After a 5 per cent excess of concentrated HCl was added, the solution was made up to 80 per cent ethanol. The precipitate which formed was separated, suspended in hot water, and an excess of FeCl<sub>3</sub> solution was added. The acidity was adjusted to *N*/6 HCl. After heating for 20 minutes on the steam bath, the precipitate was centrifuged off and washed with *N*/6 HCl. The product contained 121 mgm. of organic P.

The alcoholic filtrate was treated with FeCl<sub>3</sub> solution, and the precipitate which formed contained the remainder of the organic P of the extract.

The precipitates so obtained were combined, and attempts were made to purify them by reprecipitation. The material was suspended in 1 per cent NaOH and decomposed by digestion on the steam bath for 20–30 minutes. The Fe(OH)<sub>3</sub> was removed, the filtrate acidified, and the product re-formed by the addition of FeCl<sub>3</sub> solution. Some extraneous organic matter was removed in the filtrate, but the precipitate was brown, and the NaOH solution obtained on decomposing it was dark colored. Bromine water was added until

the color was discharged, the excess  $\text{Br}_2$  was removed by acidifying and boiling, and the cooled solution was extracted with ether. The solution was now colorless, and when  $\text{FeCl}_3$  was again added, the precipitate appeared as a pure white product, identical in appearance with ferric phytate. This material was found to be entirely free of nitrogen.

Apparently the most suitable procedure is to precipitate the organic phosphorus from the brominated extract by the addition of  $\text{FeCl}_3$  and alcohol, decompose this precipitate with  $\text{NaOH}$ , and discard the  $\text{Fe}(\text{OH})_3$ . The  $\text{NaOH}$  solution should then be further brominated, acidified, extracted with ether, and a final precipitation carried out in  $N/6$   $\text{HCl}$  by the addition of  $\text{FeCl}_3$ . Satisfactory products have been obtained in this way from several soils and also from two specimens of the soil "nucleotide" preparation which had been saved from the earlier work of Wrenshall and McKibbin (29).

A sample of the precipitate so obtained was decomposed with  $\text{NaOH}$ , and  $\text{Fe}(\text{OH})_3$  was removed as usual. Analysis showed that the solution contained 48.6 mgm. of P; the  $\text{Fe}(\text{OH})_3$  contained 63.8 mgm. of Fe, determined by the dipyriddy method (20); hence the P/Fe ratio was 0.76.

A sample of another similar preparation was washed with alcohol and ether and dried in a vacuum, when it weighed 59.7 mgm. It was decomposed with  $\text{NaOH}$ , and P and Fe were determined. The P content was found to be 9.97 per cent; the Fe, 14.4 per cent; and the ratio P/Fe, 0.69.

No analyses of ferric phytate preparations were found in the accessible literature. A specimen of ferric phytate was prepared by the addition of excess  $\text{FeCl}_3$  to an acid solution of the authentic phytin from wheat bran. The ratio P/Fe was found to be 0.71.

The possible effect of bromination was taken into account by boiling with  $\text{Br}_2$  a portion of the Na-phytate solution obtained in the previous test. The Fe-phytate which was obtained after this treatment was found to have a P/Fe ratio of 0.68.

Another sample of authentic Fe-phytate, prepared in the same way, was washed with alcohol and ether and dried in a vacuum. On heating in an air oven at  $105^\circ\text{C}$ ., the preparation decreased in weight by 17.5 per cent. This result is in agreement with the observation of Posternak (22) that phytin salts usually contain much moisture which is difficult to remove. Analysis gave the following results, expressed on the oven-dry basis: P, 15.6 per cent; Fe, 23.6 per cent; P/Fe = 0.66.

The P/Fe ratios observed in these preparations were much lower than had been anticipated. A specimen of Fe-phytate was prepared by adding to a phytin solution less  $\text{FeCl}_3$  than was required to precipitate all the phytin. The precipitate was washed with alcohol and ether and dried in a vacuum. On heating in the air oven at  $105^\circ\text{C}$ ., the loss in weight was 9.64 per cent. A portion was decomposed with  $\text{NaOH}$  and analyzed as before. The results were as follows: P, 19.1 per cent; Fe 17.3 per cent; P/Fe = 1.10. Also a portion was ignited with  $\text{Mg}(\text{NO}_3)_2$ , and the  $\text{HCl}$  solution of the ash was

analyzed. The P content was found to be 19.8 per cent; the Fe, 17.3 per cent; and the ratio P/Fe, 1.14.

The P/Fe ratio of this preparation differs greatly from those previously determined, and corresponds fairly well with the values found by titration (24). No excess of  $\text{FeCl}_3$  was present during the precipitation, and hence the product doubtless corresponds to the one formed during titration. It appears that there are two iron salts of phytin and that the product isolated from soil corresponds closely to one of them.

Ratios of P/Fe were now estimated by  $\text{FeCl}_3$  titration according to Rather (24). Sodium phytate solutions were prepared by decomposing Fe-phytate with NaOH, and corresponding solutions were obtained by decomposing the soil product. Aliquots of these solutions were acidified and rendered *N*/6 with respect to HCl, and 2 cc. of 0.3 per cent  $\text{NH}_4\text{CNS}$  was added to each. They were then titrated with standard  $\text{FeCl}_3$  solution until a brown color was produced which persisted for 5 minutes. The results obtained are shown in table 3.

TABLE 3  
*Titration of Na-phytate solutions with  $\text{FeCl}_3$*

SOLUTION TITRATED		P PRESENT	Fe ADDED	P/Fe	
		mgm.	mgm.		
1.)	Na-salt ex. soil preparation.....	1.41	1.10	1.28	1.23
2.)		1.41	1.19	1.19	
3.)	Authentic Na-phytate.....	1.47	1.32	1.11	1.20
4.)		3.51	2.74	1.28	
5.)		3.51	2.92	1.20	

Great accuracy was not attained because of the smallness of the titers, but it is evident that there is no appreciable difference between the P/Fe values of these preparations. Furthermore, these values agree with those reported by other workers. Heubner and Stadler (13) found 1.19, Rather (24) found 1.21, and these values have been accepted by later workers (4, 12).

Average values for P/Fe ratios found by analysis of preparations and by titration are given in table 4, along with calculated values for hypothetical ferric phytates. The titration values agree very well with either the hepta-ferric salt of inositol pentaphosphoric acid, assumed by Rather (24), or the octa-ferric salt of inositol hexaphosphoric acid, which seems the more probable on theoretical grounds (2). The ratio found for the ferric salt precipitated in the presence of excess phytin also approximates these calculated values. The salts precipitated with  $\text{FeCl}_3$  in excess appear to contain somewhat more Fe than the theoretical iron-saturated salt.

The Fischler and Kurten test for phytin (10) was applied to the NaOH solutions from ferric phytate and from the soil preparation. Ten milliliters of

aliquots of the solutions, each containing about 2.5 mgm. of P, were evaporated to syrupy consistency in small flasks. Each showed a greenish yellow color at this stage. A few milligrams of solid  $\text{Na}_2\text{O}_2$  and 2 drops of water were mixed into each residue. The flasks were then carefully heated over an open flame until a carmine-red color appeared and spread throughout the mixture. Intense carmine-red colors developed in both tests.

*Enzyme dephosphorylation.* A water extract of the mucosa of pig's intestine was prepared. Such an extract is said to contain both diesterases and monoesterases (17), and is supposed to be virtually devoid of phytase (21). Ten

TABLE 4  
*Comparison of various P/Fe ratios*

MATERIAL	P/Fe		
	By analysis of the Fe-salt	By titration of the Na-salt	Calculated
Soil preparation (excess Fe) . . . . .	0.72	1.23	
Authentic phytin (excess Fe) . . . . .	0.69	1.20	
Authentic phytin (excess phytin) . . . . .	1.13		
$[\text{C}_6\text{H}_5 \cdot \text{H}_4(\text{PO}_4)_3]_3 \text{Fe}_8$ . . . . .			1.24
$[\text{C}_6\text{H}_5\text{OH} \cdot \text{H}_3(\text{PO}_4)_3]_3 \text{Fe}_7$ . . . . .			1.19
$\text{C}_6\text{H}_5(\text{PO}_4)_3 \text{Fe}_4$ . . . . .			0.83

TABLE 5  
*Phosphatase dephosphorylation*

TEST SOLUTION	OR- GANIC P ADDED	INORGANIC P FOUND				DECOMPOSITION					
		0 days	3 days	6 days	10 days	3 days	6 days	10 days	3 days	6 days	10 days
		mgm.	mgm.	mgm.	mgm.	mgm. P	mgm. P	mgm. P	per cent*	per cent*	per cent*
1. Control . . . . .	0.0	0.18	0.55	0.58	0.58	....	....	....	....	....	....
2. Nucleic acid . . . . .	4.85	0.18	3.09	4.00	4.23	2.54	3.42	3.65	52.4	70.5	75.3
3. Na-salt from soil . . . . .	1.22	0.19	0.66	0.82	0.91	0.11	0.24	0.33	9.0	19.6	27.0
4. Na-phytate . . . . .	1.43	0.18	0.52	0.60	0.66	-0.03	0.02	0.08	0.0	1.4	5.6

\* Of organic P.

milliliters of this extract was added to 5 cc. of test solution, which was then adjusted to approximately pH 8.5 (phenolphthalein). One milliliter of toluene was added, and the solutions were incubated at 35°C. At intervals aliquots were withdrawn for the determination of inorganic phosphate. The test solutions contained  $\text{H}_2\text{O}$  (control), ribonucleic acid (Eastman), Na-salt of soil preparation, Na-phytate. The results are shown in table 5.

The nucleic acid was 75 per cent dephosphorylated, in accordance with the observation of Gulland and Jackson (11). The sodium phytate was hydrolyzed to a slight but appreciable extent, and the compound from soil released 27 per cent of its phosphorus under the same conditions.

A substance with considerable phytase activity was extracted from a quantity of wheat bran with five times its weight of cold water (21). To 10 cc. of test solution, 3 cc. of this extract was added, and the pH was adjusted to about 4.7 with p-nitrophenol indicator (14). The solutions were made up to 15 cc., 1 cc. of toluene was added to each, and they were incubated at 35°C. The test solutions contained H<sub>2</sub>O (control); Na-phytate; Na-salt of soil preparation; Na-phytate, previously brominated; Fe-phytate in suspension; ribonucleic acid. The results of phosphate determinations made at intervals are given in table 6.

TABLE 6  
*Phytase dephosphorylation*

TEST SOLUTION	ORGANIC P ADDED	INORGANIC P FOUND			P DECOMPOSITION			
		0 days	3 days	7 days	3 days	7 days	3 days	7 days
	mgm.	mgm.	mgm.	mgm.	mgm. P	mgm. P	per cent*	per cent*
1. Water (control).....	0.0	0.84	1.12	1.12	....	....	....	....
2. Na-phytate.....	2.86	0.88	3.45	3.56	2.33	2.44	81.5	85.3
3. Na-salt from soil....	2.44	0.87	2.84	3.30	1.72	2.18	70.5	89.4
4. Na-phytate, brom- inated.....	1.47	0.89	2.34	2.52	1.22	1.40	83.0	95.2
5. Fe-phytate.....	1.47	0.81	1.10	1.16	-0.02	0.04	0.0	2.7
6. Nucleic acid.....	2.91	0.86	3.22	3.22	2.10	2.10	72.2	72.2

\* Of organic P.

TABLE 7  
*Action of bran extract on Al-, Fe-, and Ca-phytates*

TEST SOLUTION	ORGANIC P ADDED	INORGANIC P FOUND			DECOMPOSITION			
		0 days	3 days	7 days	3 days	7 days	3 days	7 days
	mgm.	mgm.	mgm.	mgm.	mgm. P	mgm. P	per cent*	per cent
1. Control.....	0.0	1.11	1.38	1.38	....	....	...	...
2. Al-phytate.....	1.29	1.02	1.26	1.27	-0.12	-0.11	0.0	0.0
3. Fe-phytate.....	1.45	1.04	1.40	1.26	0.02	-0.12	1.4	0.0
4. Ca-phytate.....	?	1.16	2.80	2.72	1.42	1.34	...	...

\* Of organic P.

The Na-phytate, and the Na-salt of the soil preparation were vigorously attacked by the enzymes of bran extract, which released 85-95 per cent of the organic phosphorus. Apparently phosphatases were also present, as nucleic acid was 72 per cent dephosphorylated within 3 days. The Fe-phytate was extremely resistant to the action of the enzymes present.

The stability of Fe-phytate toward phytase suggested that the phytin in soils might be present in combination with iron and so be protected from enzymatic hydrolysis. The possibility was also suggested that other insoluble compounds of phytin might be formed in soil. This supposition was confirmed

when it was found that aluminum also precipitates phytic acid from solution, presumably as an Al-phytate. This product was prepared in exactly the same way as Fe-phytate, and precipitated readily from solutions as acid as pH 1.8.

A further enzyme experiment was conducted to test the action of bran extract on Al-phytate. To 10 cc. of test solution, 3 cc. of bran extract was added, the acidity was adjusted to about pH 5.0, and the whole was made up to 15 cc. One milliliter of toluene was added, and the solutions were incubated at 35°C. The test solutions contained H<sub>2</sub>O (control); Al-phytate in suspension; Fe-phytate in suspension; Ca-phytate, partly undissolved. The results of periodic analyses for phosphate are given in table 7.

The Al-phytate appears to be fully as resistant to enzyme action as Fe-phytate. Ca-phytate, which is appreciably soluble at pH 5.0, was rapidly decomposed.

### *Discussion*

The evidence which has been presented seems to establish beyond doubt the presence of phytin in the soils which have been studied. The ratio of P to Fe in the product obtained corresponded with that of authentic ferric phytate. The Na-salt responded to the Fischler and Kurten test (10), believed to be specific for phytin, and also behaved toward enzyme extracts in a manner comparable with that of authentic Na-phytate. Moreover, the procedure by which the material was obtained from soil affords additional proof of its identity. So far as the writers have been able to discover, no other organic phosphorus compound forms a ferric salt that is insoluble in *N*/6 HCl. This remark applies equally to inositol triphosphate and to inositol monophosphate, as was shown by Anderson (1, 3), who first characterized these compounds. The presence of phytin itself is not to be confused with, and, of course, does not preclude, the presence of other inositol phosphates, evidence for which was recently obtained by Yoshida (31).

The observation that Fe- and Al-phytates are virtually immune to the action of phytase in slightly acid medium affords a clue to the condition in which phytic acid is present in soils. In order to persist unchanged, phytic acid would have to be protected from the enzymes of soil microorganisms, and it now seems that this could be possible, at least in acid soils, if it were combined with Fe and Al. This need not imply that Fe- and Al-phytates as such are present in the soil. It is probable that the fixation of phytin is analogous to that of inorganic phosphate and can be effected by the active sesquioxide constituents of the soil colloids, without the latter's necessarily passing into solution. It is believed that the primary requirement for stability is that the phytic acid be rendered highly insoluble.

The extent to which phytin may accumulate under various soil conditions is unknown. The combination with Fe or Al is likely to be unstable under alkaline conditions, but Ca-phytate is relatively insoluble in alkaline solution and might persist in certain calcareous soils. The activity of phytase en-

zymes doubtless is influenced considerably by the pH of the medium, introducing another factor affecting the stability of these combinations. Presumptive evidence, however, that phytin is present in most soils is seen in the general behavior of the organic phosphorus in extraction and fractionation procedures that have been employed at various times in different localities and in the results of Dean (7), who observed organic phosphorus stable to NaOH + NaOBr in soils from several parts of the world.

### Summary

A substantial part of the organic phosphorus of the so-called soil "nucleotide" preparation was precipitated as a ferric salt in  $N/6$  HCl. The same material was precipitated from brominated soil extracts under suitable conditions and finally was obtained in an apparently pure condition. This product was free of nitrogen and contained phosphorus and iron in the same ratio as did ferric phytate similarly prepared. The sodium salt of the soil product gave the Fischler and Kurten test for phytin and corresponded to authentic sodium phytate in titration with ferric chloride and in its behavior toward enzyme extracts.

In the course of the work it was shown for the first time that phytic acid forms two distinct ferric salts, corresponding approximately to the formulas  $[C_6H_{10}(PO_4)_6]_3Fe_8$  and  $C_6H_6(PO_4)_6Fe_4$ . It was also found that a compound of aluminum with phytic acid was insoluble in acid solution.

It was observed that the preparations of ferric phytate and aluminum phytate were not subject to dephosphorylation by brain phytase in acid solution.

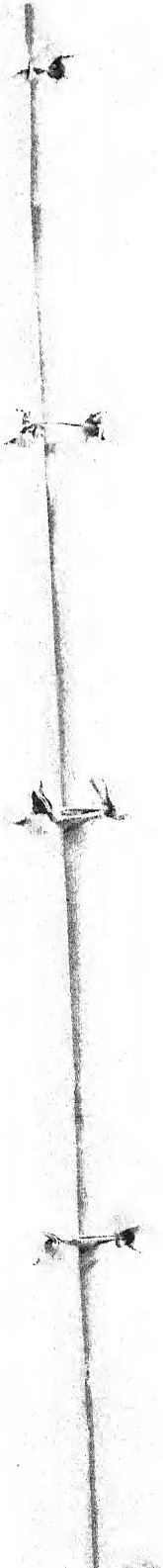
In discussing the results it was concluded that the identity of the compound obtained from soil had been established as ferric phytate. The probability that phytic acid is a common constituent of soils and that, in acid soils, it may exist in combination with iron and aluminum was pointed out.

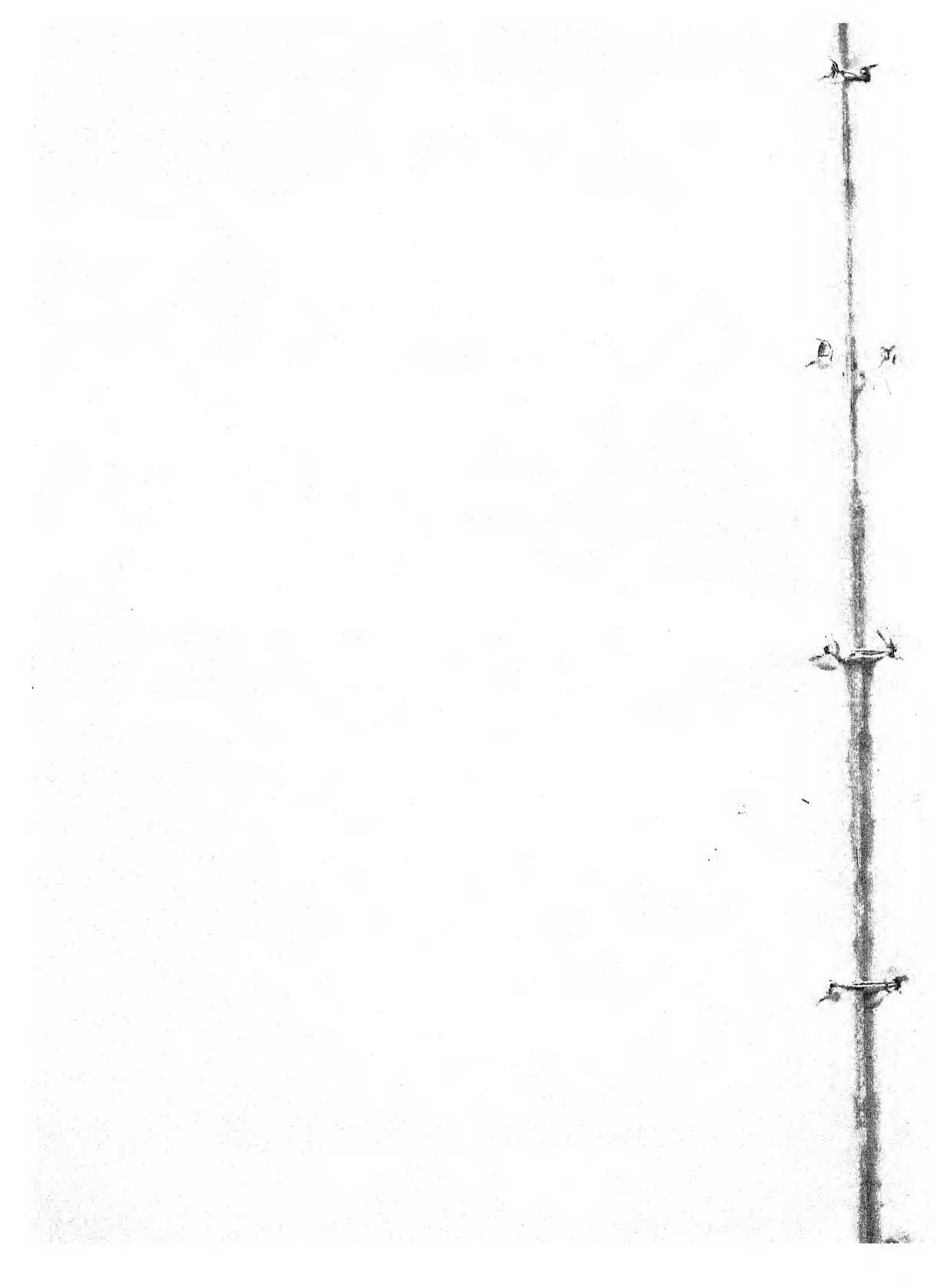
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# DETERMINATION AND BEHAVIOR OF FERROUS IRON IN SOILS<sup>1</sup>

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In literature concerning ferrous iron in soils there has been a controversy on three points: (a) Does ferrous iron enter into base-exchange reactions? (b) Does the downward movement of iron in soils such as podzols take place in ferrous condition? (c) Is ferrous iron present in large quantities in ordinary healthy soils? In connection with the last point, Willis and Piland (6) put forward a hypothesis that the intake of iron by plants depends on the oxidation-reduction potential of the soil, and they suggest that the absorption of iron by plants is influenced by copper because this element affects this potential. Kliman (3) asserts that plants reduce iron and absorb it in the divalent form.

From these statements one can infer that in soils there is a dynamic equilibrium between the ferrous and the ferric iron, an equilibrium which is influenced by the oxidation-reduction potential of the soil. This hypothesis suggests the presence of ferrous iron in soil.

The work reported was undertaken in an attempt to elucidate some of these points, as well as to determine some other properties of soil iron.

## LABORATORY INVESTIGATIONS OF SOIL FERROUS IRON

The earlier methods of determining inorganic ferrous iron in soils are open to criticism on the grounds that they depended on reduction of potassium permanganate solution, which is not a specific reaction for inorganic ferrous iron. In the work here presented the ferrous iron was determined according to the method suggested by Ignatieff (2). By this method the soil samples are extracted with 3 per cent aluminum chloride solution, and the ferrous iron of these extracts is determined by means of a dipyriddy reagent. The properties of dipyriddy and its formation of a red complex with ferrous iron have been discussed in the paper cited.

Some further work was carried out to test the value of aluminum chloride solution as an extractant reagent. It was shown (table 1) that very much larger quantities of ferrous iron can be extracted from soils with aluminum chloride solution than with water or even with such solutions as ammonium chloride or potassium chloride.

In choosing an extractant it is very important to select a reagent that will

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not cause the reduction of iron. This was a difficulty encountered by Morison and Doyne (4) when they tried to use dilute HCl for extracting ferrous iron from soil: iron was reduced by dilute HCl in the presence of organic matter.

It was proved experimentally that aluminum chloride solution does not cause the reduction of iron during the short time necessary to make the extraction, despite the fact that as a result of hydrolysis 3 per cent  $\text{AlCl}_3$  solution would contain HCl. Ferrous iron was either absent from these extracts or present in very small quantities. If samples of the same soil were kept under water for varying periods of time ranging from a few hours to 2 weeks and then extracted with aluminum chloride solution, increasing quantities of the divalent

TABLE 1

*Amounts of ferrous iron extracted per gram of moist gray wooded soil by 0.56 N solutions*

EXTRACTANT	pH OF SOLUTION	Fe''
		<i>gamma</i>
Water.....	7.40	traces
KCl solution.....	6.18	129
$\text{NH}_4\text{Cl}$ solution.....	5.92	142
$\text{AlCl}_3$ solution.....	2.92	443

TABLE 2

*Ferrous iron extracted with  $\text{AlCl}_3$  solution from black soil incubated under water and under  $\text{AlCl}_3$  solution*

*Gamma of ferrous iron per gram of soil*

	HOURS					
	0	4	20	46	71	340
Black soil incubated in 3 per cent $\text{AlCl}_3$ solution.....	0.8	1.2	2.1	3.3	4.3	35.0
Black soil incubated in water.....	0.8	1.1	1.5	1.7	2.6	542.4

iron were extracted, depending on the length of time the samples had been submerged. When other samples of the same soil were kept under aluminum chloride solution instead of water, the quantities of ferrous iron in the extract increased gradually, but the reduction of iron was much less rapid after 71 hours in soil samples under aluminum chloride solution than in those kept under water. The results of the experiment are presented in table 2.

It will be noted that the increase of ferrous iron in the extracts from the soil submerged under aluminum chloride does not take place to any appreciable extent until the soil sample has remained in aluminum chloride solution for a great number of hours.

It was of importance to ascertain what proportion of the ferrous iron in a soil soluble in a 3 per cent aluminum chloride solution could be brought into solution on the first extraction. Samples of soil from a glei horizon high in

ferrous iron were extracted five times with 3 per cent solution of aluminum chloride, and the content of ferrous iron in these extracts was determined. The results of the experiment are presented in table 3. These figures show that 80 per cent of the ferrous iron is brought into solution from the soil on the first extraction.

It was realized that ferrous iron compounds are fairly readily oxidized, especially when these are in solution. From a survey of the literature, however, it was impossible to come to any definite conclusion as to the stability of the divalent iron in soil. If the ferrous iron in soil were stable it would be

TABLE 3

*Consecutive extractions of soil samples of a glei horizon by 3 per cent  $AlCl_3$  solution*

	Fe'' PER GRAM MOIST SOIL	PER CENT EXTRACTED
	<i>gamma</i>	
1st extraction.....	435	80
2nd extraction.....	67	12
3rd extraction.....	20	4
4th extraction.....	13	2
5th extraction.....	12	2

TABLE 4

*Effect of storage of samples on the ferrous iron content of the soil soluble in a 3 per cent  $AlCl_3$  solution*

	Fe'' PER GRAM OVEN-DRY SOIL	
	At time of sampling	48 hours after sampling
	<i>gamma</i>	<i>gamma</i>
Peat abutting glei horizon.....	913	304
	555	724
Glei horizon.....	244	37
	640	259

possible to collect the samples in the field and carry out the determination in the laboratory, as is the usual practice in the determination of other elements in soil samples. The experimental results presented in table 4 demonstrate, however, that the ferrous iron content of soil samples changes on storage even during a short time.

The ferrous iron content of different soil samples was determined in the field immediately after sampling, and the determinations were repeated on the samples 48 hours later in the laboratory. The samples had been kept in sealed containers. It will be noted that the ferrous iron content in most of the samples had decreased very markedly; however, in one sample of peat which contained about 317 per cent of moisture the ferrous iron concentration

actually increased. From these results it became evident that for reliable estimation of divalent iron in soils it is imperative to carry out the determinations in the field at the time of sampling.

It was thought that the ferrous iron in soil samples might be rapidly oxidized during the process of extraction, and therefore some samples were extracted with aluminum chloride solution under a protective covering of mineral oil. This experiment showed that small quantities of ferrous iron were oxidized when no oil was used to cover the surface of the extracting liquid. As the quantities of ferrous iron oxidized are small, however, and the use of oil adds to the difficulty of determination, especially when carried out in the field, oil was generally not used in the determinations.

It was reported in a previous paper (2) that the reduction of iron in solutions would be caused by exposing such solutions to light. It was suspected that

TABLE 5

*The reduction of iron of a soil extract in buffer solution at pH 4.6 and  $\alpha\alpha$  dipyridyl when exposed to sunlight*

AlCl<sub>3</sub> solution used as soil extractant

TREATMENT	Fe'' IN GAMMA PER 10 CC. OF EXTRACT		
	Before treatment	After treatment	After reduction with hydroquinone
Exposed to sunlight:			
15 minutes.....	5.6	21.0	54.6
25 minutes.....	5.6	29.4	56.0
30 minutes.....	5.6	30.8	54.6
36 minutes.....	5.6	39.2	58.8
Kept in darkness.....	5.6	8.4	54.6

great errors may result if aluminum chloride solution extracts of soil containing both ferric and ferrous iron are added to the buffer of pH 4.6 containing  $\alpha\alpha$  dipyridyl for the determination of ferrous iron and then left exposed to sunlight. The experimental results presented in table 5 clearly indicate that such procedure could indeed be responsible for grave errors.

It is interesting to note (table 5) that the initial content of ferrous iron in 10 cc. of soil extract was 5.6 gamma, whereas the ferric and ferrous iron amounted to 56 gamma. The iron extracted from the soil (last column, table 5) remained fairly constant but the ferrous iron increased from 5.6 to 39.2 gamma after 36 minutes' exposure to sunlight. These data indicate that some of the ferric iron had been reduced as the result of exposure to sunlight. In contrast, the sample of soil extract kept in darkness showed only 8.4 gamma of ferrous iron compared with 39.2 gamma for the exposed sample.

It was of great interest to ascertain whether sunlight caused the reduction of iron in the soil itself. The figures in table 6 demonstrate that reduction of

iron in soil can be brought about by sunlight. It would appear that the temperature was not the factor responsible for the differences obtained. The experimental results with soils suspended in aluminum chloride solution and

TABLE 6  
*Effect of sunlight on iron content of soil in  $AlCl_3$  solution exposed to sunlight*

	IRON PER GRAM OVEN-DRY SOIL		TEMPERATURE OF $AlCl_3$ SOLUTION °C.
	Fe <sup>++</sup> <i>gamma</i>	Fe <sup>+++</sup> <i>gamma</i>	
Exposed to sunlight:			
0 minutes.....	3.4	11.1	26
15 minutes.....	7.7	15.4	..
30 minutes.....	10.7	13.7	27
60 minutes.....	15.4	16.2	29
95 minutes.....	17.1	14.5	27
130 minutes.....	20.5	14.5	
Soil kept in darkness for duration of experiment..	3.4	14.9	22
Soil exposed to heat equivalent to that of the sun but not to the light of the sun for duration equivalent to that of the experiment.....	6.0	17.1	28

TABLE 7  
*Ferrous iron in seepage water from black, gray wooded, and glei soils submerged under water*

WATERLOGGING PERIOD	SOIL	Fe <sup>++</sup> PER CUBIC CENTI- METER OF PERCOLATE	Fe <sup>++</sup> PER GRAM OVEN-DRY SOIL
<i>days</i>		<i>gamma</i>	<i>gamma</i>
1	Glei	0.4	....
2	Glei	0.4	....
6	Glei	0.7	....
8	Black	4.0	....
	Gray wooded	13.0	....
18	Glei	1.0	....
	Glei	1.0	....
20	Black	18.0	....
	Gray wooded	39.0	....
37	Glei	1.2	....
	Black	53.0	1961
	Gray wooded	62.0	1123
	Glei	2.0	265

exposed to sunlight are more definite than those obtained with untreated soil, but the data for the untreated soils show a similar trend. Different types of soils were used and a similar success was not achieved in all cases.

The data presented in table 1 suggest that ferrous iron enters into the base-



exchange complex of the soil. In table 7 are reported the results of an experiment in which soils were submerged under water in percolators. At different periods of time the water was allowed to pass out of the percolators and was analyzed for ferrous iron. At the end of 37 days the percolates and the soils were analyzed, giving the results shown in the table. From the figures it is evident that the water percolating through the soil could bring into solution only a small fraction of the ferrous iron present in the soil.

In an experiment, the results of which are shown in table 8, the effect of submerging soil under water with drainage and without drainage is compared. About 800 cc. of water was allowed to drain away daily from those percolators in which drainage was permitted. It is seen that the total amount of ferrous iron that is rendered water soluble is greater in the percolators that were not

TABLE 8

*Effect of drainage on some of the properties of water-logged soil;  $E_h$  values of the percolates, the amount of water passing through the percolators, and total water-soluble ferrous iron in each percolator*

PERCOLATOR NUMBER	AMOUNT OF WATER PASSED THROUGH SOIL IN 16 DAYS	Fe <sup>++</sup> PRESENT AFTER 16 DAYS		$E_h$ VALUES OF THE PERCOLATES
		In soil solution plus amount washed out of soil	In soil solution	
	cc.	gamma	gamma	millivolts
1	10,000	10,500		+111
3	14,000	11,200		+166
5	15,000	13,300		+166
2			22,500	+98
4			15,500	+82
6			14,000	+103

Note: Gray wooded soil was used in all percolators.

In percolators 1, 3, 5, water was allowed to pass in amounts indicated.

In percolators 2, 4, 6, soil remained undrained.

drained than in those allowed to drain. The  $E_h$  values (table 8) were measured and proved to be higher in the percolates from the drained soils. From table 9 it is seen that ferrous iron formed rapidly in unsterilized soil under water but not in steam-sterilized waterlogged soil maintained under aseptic conditions. The ferrous iron content of steam-sterilized soil is much greater immediately after sterilization than that of the unsterilized soil.

A rise in aluminum chloride soluble ferrous iron takes place during autoclaving. This is well demonstrated in table 10. Black soil, in crocks covered with paper, was sterilized in an autoclave. The ferrous iron content of the soil was determined before sterilization, immediately after sterilization, and at different intervals after sterilization. The analyses were made on different crocks to prevent any interference from organisms. It appears that steam sterilization causes a rise in aluminum chloride soluble ferrous iron, and some of

this ferrous iron persists in the soil for a considerable time (tables 9 and 10). There is a fairly rapid disappearance of this divalent iron during the first few hours, but within several days the ferrous iron content of the soil becomes fairly constant. It is interesting to note that even after 28 days (table 10) there is as much as 8.2 gamma of ferrous iron per gram of dry matter in the black soil, which normally does not contain more than 1.0 gamma of ferrous iron per gram of dry matter.

TABLE 9

*Ferrous iron content of black soil under water as affected by steam sterilization and incubation for long periods of time*

INCUBATION PERIOD	Fe'' PER GRAM OF SOIL	
	Unsterilized	Autoclaved
<i>hours</i>	<i>gamma</i>	<i>gamma</i>
0	0.7	102
5	...	64
20	0.7	70
44	1.4	82
99	18.0	82
363 (15 days)	302.0	19
1035 (43 days)	2100.0	25

TABLE 10

*Effect of steam sterilization on ferrous iron in black soil*

TREATMENT	Fe'' PER GRAM OF OVEN- DRY SOIL
	<i>gamma</i>
Before sterilization.....	0.6
Immediately after sterilization.....	65.0
1 day after sterilization.....	11.7
3 days after sterilization.....	14.9
9 days after sterilization.....	9.7
28 days after sterilization.....	8.2

#### FIELD INVESTIGATIONS OF FERROUS IRON IN SOME ALBERTA SOILS

It has been previously mentioned that the ease with which ferrous iron in soil was oxidized necessitated carrying out the determinations in the field. The results of experiments just quoted definitely indicated that the soil and soil extracts must be carefully protected from light and especially from sunlight. For this reason all vessels used were covered with black paper.

The field method of determining ferrous iron in soils is the same in principle as that described in a previous paper (2). Twenty grams of soil were extracted with 50 cc. of 3 per cent aluminum chloride solution. The extraction was performed by thoroughly stirring the soil suspension with a glass rod. The

suspension was then centrifuged in a hand centrifuge. Measured quantities of the supernatant clear liquid were pipetted into 15 to 20 cc. of buffer solution of pH 4.6 containing  $\alpha\alpha$  dipyridyl. The color developed was compared against a standard. Samples were also taken for determining the moisture content of the soil.

It must be understood that the ferrous iron content of soils reported in this paper refers to the iron soluble in a 3 per cent solution of aluminum chloride; this does not necessarily represent the total ferrous iron content of the soils. Furthermore, it must be remembered that the first extraction of soils by aluminum chloride solution removes only about 80 per cent of the ferrous iron soluble in this extractant. Alpha alpha dipyridyl will not form a red complex with ferrous iron attached to a nitrogen atom. Compounds of this nature, if they existed in the soil, would not be determined by the method outlined.

TABLE 11  
*AlCl<sub>3</sub>-soluble ferrous iron in the different horizons of a peat bog*

DESCRIPTION OF HORIZONS	DEPTH	Fe'' PER GRAM OVEN-DRY SOIL	MOISTURE, DRY BASIS
	<i>inches</i>	<i>gamma</i>	<i>per cent</i>
Peat.....	6-12	15	427
Peat.....	18-24	360	400
Peat just overlying the loam.....	24-25	954	354
Dark brown heavy silty loam high in organic matter.....	25-26	318	82
Light brown heavy silt loam.....	26-28	276	32
Blue-gray clay loam.....	28	244	16

It is doubtful, however, whether iron compounds similar to haemoglobin exist in the soil (1).

Genetically the soils termed "gray wooded" in the literature of the soils department of the University of Alberta are similar to podzol soils. The black soils of Alberta closely resemble the chernozems. Dozens of samples were analyzed in the field at widely divergent points, covering both gray wooded and black soils. Analyses were carried out on samples from different depths and from soils under different types of vegetation.

It was also possible to analyze samples from peaty swamps and samples of black soil that had been submerged during the spring thaw and rains. Determinations were also carried out on the waters draining away and overlying these wet localities.

The analyses for the fertile black and gray wooded soils in dry situations are not tabulated because no outstanding results were obtained. One may summarize the data by saying that healthy gray wooded and black soils to a depth of about 2 feet appeared to contain only the smallest amount of ferrous iron, not more than 2 to 3 p.p.m. There were samples of both classes of soil

in which no ferrous iron or only traces were found. Analyses of soils under different vegetation, such as grain crops, pasture, or woodland, did not reveal any definite differences in ferrous iron content. There were indications, however, that some of the gray wooded soil samples contained slightly greater amounts of ferrous iron than did the black soils.

In table 11 are shown the figures obtained on the different horizons of a peat bog. It will be noted that from 18 inches downward the concentration of ferrous iron is high and that it is much higher in the peaty horizon immediately above the loam than in the silty loam horizon. This very high concentration can be partly explained by the low specific gravity of the peat: the results are reported on the basis of 1 gm. of dry matter; since 1 gm. of peat has a much greater volume than 1 gm. of loam, peat would provide a much larger surface for absorption of ferrous iron.

TABLE 12

*AlCl<sub>3</sub>-soluble ferrous iron of the different horizons of a peat bog and of a gray wooded soil*

DESCRIPTION OF HORIZONS	DEPTH	Fe'' PER GRAM OVEN-DRY SOIL	MOISTURE, DRY BASIS
	<i>inches</i>	<i>gamma</i>	<i>per cent</i>
Peat.....	15	0	376
Peat just overlying loam.....	27-28	262	317
Brown loam.....	28-30	112	45
Blue-gray clay loam.....	Below 30	222	18
Gray wooded soil (50 yards from peat bog).....	0-6	0	22
	12-18	28	14

In table 12 are presented the results of analyses on samples of soils taken in two localities some 50 yards apart, one in a peat bog, and the other on higher ground representing the gray wooded soil. The high ferrous iron content of the glei horizon clearly indicates the effect of soil moisture on the ferrous iron content of the soil. The two locations were chosen so close together in order that climatic conditions such as rainfall and temperature might be as nearly alike as possible. It was also noted that the physical composition of the inorganic fraction of the soil in the two localities was similar. The peat bog was formed in a basin, and the gray wooded soil was developed on the rising land forming the sides of this basin. The moisture content of the samples of the peat overlying the glei horizon is very high.

It was of interest to ascertain the effect of waterlogging of soil that under normal conditions is dry. A field in the black soil zone was selected, and samples were taken in three locations. Location A was chosen on high ground on which water had not remained during the spring thaw and subsequent heavy rains. Location B was in a shallow basin 5 feet away from the water collected in the basin during the spring thaws. Location B may have been under water in the earlier part of the spring, but it was not covered by water

at the time of sampling. Location C was under water when the soil was sampled. A month later samples at location B were taken again. The results of the analyses are given in table 13.

These figures show that the soil on high ground which had not been water-logged, although very moist, contained only traces of ferrous iron. The soil

TABLE 13

*AlCl<sub>3</sub>-soluble ferrous iron of black soil samples taken in a field at locations with different moisture contents*

DESCRIPTION OF SAMPLE	DEPTH	Fe'' PER GRAM OVEN-DRY SOIL	MOISTURE, DRY BASIS
	<i>inches</i>	<i>gamma</i>	<i>per cent</i>
Location A—high ground:			
Black loam.....	0-6	traces	50
Brown clay loam.....	18-24	traces	27
Brown clay loam.....	30-36	traces	27
Location B—100 yards from A, 5 feet from slough water:			
Black loam.....	0-6	32.0	51
Brown clay loam.....	18-24	5.3	39
Location C—5 yards from B, under 6" of water:			
Black loam.....	0-6	244	56
Location B—31 days later:			
Black loam.....	0-6	7.7	37
Brown clay loam.....	12-18	15.1	35
Brown clay loam.....	30-36	.9	30

TABLE 14

*AlCl<sub>3</sub>-soluble and water-soluble ferrous iron in different soils and in slough waters*

FERROUS IRON PER GRAM OF SOIL EXTRACTED WITH AlCl <sub>3</sub> SOLUTION	FERROUS IRON EXTRACTED WITH WATER
<i>gamma</i>	<i>gamma</i>
Glei soil..... 532	From 1 gm. of glei soil..... 35
Oven-dry soil from slough bottom..... 244	In 1 cc. of slough water..... 0.3
Oven-dry peat..... 513	In 1 cc. of water from peat bog..... Slight trace

at location B probably had been covered by water; and although its moisture content did not appear to be very much higher than that of location A, its aeration must have been in a poorer state than that of location A. It is interesting to note that the ferrous iron content of the top 6 inches of black soil (location B) is much higher than that of the clay loam subsoil 12 to 18 inches below it. This would indicate that there was not very rapid movement

of ferrous iron in this type of soil. Location C was under water. The soil sample from C contained a large quantity of ferrous iron.

When location B was sampled 31 days later and the soil had the opportunity to dry to a certain extent and become better aerated, the analyses show that ferrous iron in soil is oxidized fairly rapidly. These results confirm the finding presented in table 4.

From the following experiment it is evident that under field conditions ferrous iron is not easily extracted from the soil by water. Different samples of the glei horizon high in ferrous iron were extracted with  $\text{AlCl}_3$  solution and also with distilled water. Analyses were made of the water and the peat in a peat bog and the water and the soil of the bottom in a slough. The results of the determinations are presented in table 14.

Only one-fifteenth of the quantity of ferrous iron was extracted from the glei soil by water as by aluminum chloride solution. The water of the peat bog and of the slough contained only small traces of ferrous iron although in contact with materials containing large quantities of iron in the divalent form. It must be emphasized that the slough was very shallow, and the water from the peat bog was squeezed out of the peat. It would thus appear that this low concentration of the ferrous iron in water was not due to dilution.

#### DISCUSSION

The first six tables deal with improvement of the method for the determination of ferrous iron and present some interesting properties of the soil ferrous iron.

The results shown in table 1 clearly indicate that ferrous iron enters the base exchange complex of the soil, from which it can be displaced by different salts. The more efficient action of aluminum chloride in the displacement of the divalent iron may be due to the free  $\text{HCl}$  formed on hydrolysis of aluminum chloride or the greater activity of the  $\text{Al}$  ion.

It has been pointed out that the results presented in table 2 indicate that aluminum chloride itself does not stimulate the production of ferrous iron in soils.

The experiment reported in table 4 demonstrates that the large quantities of ferrous iron in waterlogged soil, if exposed to air, are very quickly oxidized.

In tables 5 and 6 the effect of sunlight on soil iron is shown. It was possible to demonstrate that the reduction of soil iron is stimulated by the light rays of the sun and not by the heat of the sun. This finding is of great interest, as it suggests an important catalytic oxidative action of soil iron. Russell and Smith (5) had shown that ferric oxide could oxidize to a certain extent even such a stable compound as ammonia. It is hard to explain why this marked increase in ferrous iron concentration of the soil on exposure of the sample to sunlight was not obtained with every soil used. It is possible that in soils with different contents of organic matter the intensity of oxidative processes may vary. The ferrous iron concentration would be built up in

those soils in which the rate of reoxidative reactions is slow, whereas no appreciable change would be noted in the ferrous iron concentration of a soil exposed to sunlight if the reoxidation in it took place at a rapid rate. The very much higher ferrous iron concentration obtained with the soil suspension in aluminum chloride solution as compared to the concentration of the divalent iron in untreated soil when the suspension and the untreated soil were exposed to the sunlight, may possibly be explained by the slowing down of oxidative processes in the soil by aluminum chloride solution.

The results in table 5 show that in using dipyrldyl as a quantitative reagent for ferrous iron determination, definite precautions have to be taken if the solution under investigation contains both ferric and ferrous iron. The exposure to light of such a solution, if it contains any oxidizable material, will cause the reduction of ferric iron. In a previous publication (2) it was shown that ferric iron in a solution containing only  $\alpha\alpha$  dipyrldyl if exposed to light was reduced. This source of error has not been previously mentioned by investigators using dipyrldyl.

The results in table 2 show that when the black soil is waterlogged the reduction of iron takes place slowly at first and then the process is rapidly increased. The data in tables 7 and 8 indicate that when the soil contains large quantities of ferrous iron a slow downward movement of it in solution can take place. Similar trends were obtained with other soils of different organic matter contents.

The analyses of the soils in the percolators at the end of the experiment show that the ferrous iron (soluble in aluminum chloride solution) of the submerged soils—drained or not drained—was approximately equal; it is therefore difficult to explain why more ferrous iron was rendered water-soluble in the percolates that were not drained than in those allowed to drain. It is possible that the oxygen introduced into the soil solution by the incoming water may have been responsible for these results. It is also of interest to note that the  $E_h$  values of the percolates from the drained soils were higher than those of the percolates from the undrained soils.

The  $E_h$  values indicate that the oxidation-reduction system operating in the submerged soil has a stronger reducing power than the ferrous-ferric system. The  $E_o$  potential of an equimolar mixture of  $Fe''' + Fe''$  system is +750 millivolts at 18°C. as referred to a normal hydrogen electrode.

The results of the experiment reported in table 9 show that the reduction of iron in waterlogged soils is largely a biological process.

The data presented in tables 9 and 10 undoubtedly show that when a soil is autoclaved there is a great increase in the ferrous iron soluble in aluminum chloride solution. It is possible that autoclaving the soil merely makes the ferrous iron already present in the soil more soluble by breaking down some of the more complex iron organic compounds which may be insoluble in aluminum chloride solution.

This latter explanation may be a plausible one, but it is not so likely as the

one based on the theory of reduction of soil iron. It will be noted that during autoclaving there is a rapid formation of ferrous iron soluble in aluminum chloride solution; a few hours after autoclaving, the concentration of this ferrous iron has fallen rapidly. This rapid fall in concentration of divalent iron may be explained by reoxidation of the iron. It is unreasonable to suppose that ferrous iron as easily oxidized as has been demonstrated in tables 9 and 10 could have persisted in a healthy soil, even if it were to be stabilized in some complex organic combination. Furthermore, in experiments not reported in this paper, it was found that when iron salts were autoclaved in contact with organic matter such as agar-agar, the reduction of iron to the divalent form took place. A hypothesis that the reduction of iron takes place when soil is autoclaved is therefore justified. It must be admitted, however, that after autoclaving, certain quantities of the divalent iron persisted in the soil for a considerable time. Whatever view is accepted, the fact remains that autoclaving of soil causes a high concentration of more soluble ferrous iron. These results are especially interesting because autoclaved soil has been used extensively as a medium in the study of soil bacteria in general, and particularly of those causing plant diseases. It is essential that further work be done on the effect of ferrous iron on soil microflora. These results also suggest that the  $E_h$  values of the soil may be lowered during autoclaving. The lowering of  $E_h$  values has been observed during steam sterilization of milk. It is of importance to note that in the experiment on autoclaving the soil in crocks (table 10), 8.2 gamma of ferrous iron per gram of dry soil was found present 28 days after sterilization. This concentration (8.2 gamma) of ferrous iron is equivalent to approximately 50 pounds of ferrous sulfate per acre (the calculation is based on a 6-inch depth of soil), the usual rate of application of the substance when it is used as a corrective on infertile soils. It has already been pointed out that all the analyses carried out indicate that healthy soils with drainage contain very small quantities of ferrous iron soluble in aluminum chloride solution, and some samples contained no ferrous iron.

The results in table 11 are interesting, as they show the ferrous iron concentrations in the different horizons of a peat bog. One explanation for the very high concentration of ferrous iron in the lower horizon of peat has already been suggested in the experimental section of this publication, but no explanation has been offered for the origin of ferrous iron in the peat layers. It is possible that the ferrous iron present in the peat layers may have originated from the iron compounds present in peat. This suggestion, however, is improbable, because the 6- to 12-inch horizon which has a moisture content of 427 per cent, contains only 15 gamma of ferrous iron per gram of peat, whereas with the lower strata the closer they are situated to the clay loam glei horizon, the greater amount of ferrous iron they contain. It would seem more likely that the divalent iron found in peat has been absorbed by it from the glei horizon.

It is interesting to note that the moisture content of the glei horizon below



the 28-inch depth is low: the maximum water-holding capacity of this soil is about 34 per cent, and yet the moisture content of the horizon was about 16 per cent. Notwithstanding this low moisture content, the ferrous iron concentration of the horizon was high at the time of sampling, in August. It may be that the ground waters during the spring had waterlogged the soil, large quantities of ferrous iron were produced, and some of this may have been transported into the peat by the upward movement of the ground waters. Later during the summer the ground waters receded, leaving the glei horizon fairly dry. The peat, however, held large quantities of water throughout the summer, creating an air-proof layer which would preserve anaerobic conditions and prevent the oxidation of ferrous iron in the glei horizon.

There is an alternative explanation for the presence of ferrous iron in the glei horizon of low moisture content. It is possible that the water-saturated layer of peat had created anaerobic conditions in the glei horizon in which the iron was reduced. Whatever explanation is taken for the reduction of iron in the glei horizon it is of interest to observe that ferrous iron can persist in a stratum of soil of fairly low moisture content if the upper layers of soil are of such a nature that they prevent the free passage of air.

This observation is again well demonstrated by the results presented in table 12. The samples were purposely taken at locations which were very close to one another. In one case the glei horizon under water-saturated peat contained large quantities of ferrous iron, and in the other case the horizon of gray wooded soil contained only the smallest quantities of the reduced iron.

The formation of large quantities of divalent iron in soil which normally contains only traces of it, is well illustrated in table 13. The results in this table also show that the ferrous iron in the soil is fairly rapidly reoxidized if soil aeration improves. This has already been pointed out in the discussion of the results of table 4.

The solubility of ferrous iron in soil water has been frequently discussed. The data presented in table 14 definitely indicate that ferrous iron present in the soil is not easily brought into solution by water. The ferrous ion is fixed by the soil in the same manner as any other basic ion.

It would thus appear that in soil the downward movement of iron in the divalent form would be very slow, and that large quantities of the soil iron would have to be reduced before any appreciable quantities of iron are moved in that state.

#### SUMMARY

An improved method for the determination of ferrous iron in soil has been outlined, and sources of possible error have been discussed.

The ferrous iron content of soils in the black and the gray wooded soil zones in Alberta was determined. The effect of soil moisture on the ferrous iron content of the soil was studied. Some experiments to elucidate the properties of soil ferrous iron were carried out. It was found that

Well-drained, healthy soils investigated, contained very small quantities of ferrous iron. In some samples the presence of the divalent iron was not detected.

Under waterlogged conditions large quantities of ferrous iron were produced rapidly, after an initial lag of two or three days. This is mainly a biological process.

The soil ferrous iron is not readily brought into solution by water, but by the use of salts the ferrous iron can be displaced from the soil. Aluminum chloride proved to be the most efficacious for this purpose. This would indicate that ferrous iron enters into the base exchange complex of soil.

Under anaerobic conditions soil water could bring into solution quantities of ferrous iron, but much larger amounts of the divalent iron were held by the soil base exchange complex.

The  $E_h$  values indicate that the activity of the reducing agents present in waterlogged soil is greater than the reductive activity of ferrous iron.

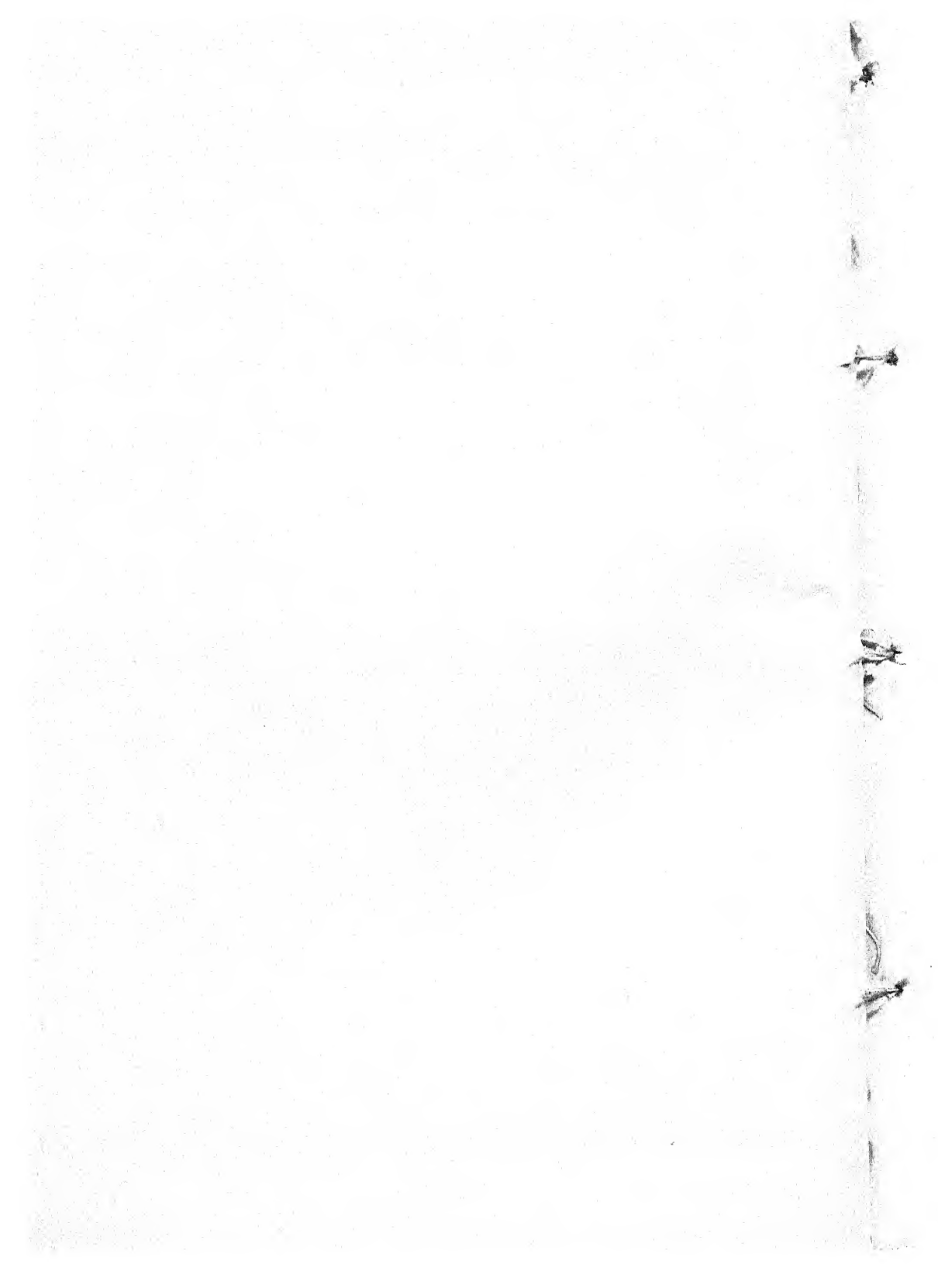
Ferrous iron in soil becomes readily oxidized if aeration of the soil is improved.

Ferrous iron is formed when soil is steam-sterilized, and some of this divalent iron persists in the soil for a considerable length of time.

Sunlight facilitates the reduction of iron in some soils and soil extracts. There is a possibility that iron acts as a catalyst, and sunlight as a source of energy in the oxidation processes in soils.

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# SORPTION OF POTASSIUM AND AMMONIUM BY SOILS AS INFLUENCED BY CONCENTRATION AND THE DEGREE OF BASE SATURATION<sup>1</sup>

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Many of the agricultural soils in high rainfall regions of the Hawaiian Islands are in a low state of base saturation. Because of the high rainfall, which, in the most humid districts, is above 200 inches annually, a knowledge of the abilities of these soils to retain potassium and ammonium fertilizers becomes important to the sugar cane grower. It has been shown by Peech and Bradfield (6) and more recently by Peech (7) that the abilities of soils to sorb potassium from neutral potassium salts decrease as the degree of base saturation decreases. It seems probable that sorption of ammonium is similarly affected by the state of saturation. In view of these considerations it was deemed appropriate to study the effect of the degree of base saturation of these soils upon their abilities to sorb potassium and ammonium salts.

It is a common practice in Hawaii to apply nitrogen and, at times, potassium to sugar cane by dissolving the fertilizer in the irrigation water prior to its application to the field. Since an irrigation of sugar cane involves from 5 to 10 acre-inches of water, the resulting dilution of the salt is great. The effect of such dilution upon the sorption of the salts by Hawaiian soils has not previously been studied. A second objective of this work, therefore, was to determine the influence of concentration upon the sorbability of potassium and ammonium by the soil.

## EXPERIMENTAL PROCEDURE

Two soils were selected for the study: one, a Hilo-coast soil from the Island of Hawaii, is representative of soils of the more humid districts where irrigation is unnecessary; the other, from the Aiea region of the Island of Oahu, is typical of large areas of drier, irrigated sugar cane lands of the islands. Both soils are residual and lateritic in nature. Certain other characteristics of these soils are listed in table 1.

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<sup>2</sup> Assistant chemist. The writer is indebted to L. A. Dean for the helpful suggestions and criticisms tendered.

Substantial quantities of the two soils were pulverized to pass a 1-mm. screen. They were then saturated with calcium by leaching with a solution (pH 6.8) which was 0.5 *N* with respect to calcium acetate and 0.1 *N* with respect to calcium chloride. The chloride was included to simplify the testing of the washed soil. After treatment with the calcium solution, the soils were washed with water until free of chlorides. As a result of this treatment the pH of each soil was 7.4. One-hundred-gram portions of these calcium-saturated soils were then adjusted to various degrees of saturation by electro-dialysis. In the case of the Hilo-coast sample, soils at eight stages of base saturation were prepared in this manner; with the Aiea soil, three. Since ammonium is applied almost exclusively by the plantations as the sulfate in fertilization and potassium as the chloride, these salts of the cations were employed in the study.

The abilities of the two soils at the various degrees of calcium saturation to sorb potassium and ammonium were measured by the following procedure: Duplicate 5-gm. samples of the air-dried prepared soils were shaken with

TABLE 1  
*Description and some chemical characteristics of the soils studied*

SOIL	DESCRIPTION	ORGANIC MATTER	ULTIMATE pH	EXCHANGE CAPACITY*	EXCHANGE CAPACITY DUE TO ORGANIC MATTER
		<i>per cent</i>		<i>m.e./100 gm.</i>	<i>per cent</i>
Hilo-coast . . . . .	Light brown clay	15.6	4.3	41.2	76.6
Aiea . . . . .	Red clay	4.7	4.3	17.4	27.0

\* pH (Ca-soil) = 7.4.

250 cc. of potassium chloride (or with ammonium sulfate) in an end-over-end shaker for 1 hour. The suspensions were allowed to stand 16 to 18 hours. They were then poured on filters in 8-cm. Büchner funnels and the solutions drawn through under suction. Additional units of 500 cc. of the fresh solutions were then percolated through the soils under gravity, the rate of percolation being so adjusted that the process required 16 to 20 hours. Upon completion of the percolation, the soils were washed with 80 per cent ethyl alcohol until free of soluble salt. Where potassium was the sorbed cation, the amount was determined by subsequent displacement with a normal ammonium acetate solution adjusted to pH 6.8. The potassium thus displaced was determined by the volumetric sodium cobaltinitrite method of Volk and Truog (9). The sorbed ammonium was determined by distillation with magnesium oxide, corrections being made for any breakdown of organic soil nitrogen in the process by similarly distilling samples of the untreated completely electro-dialyzed soils. In order to ascertain the effect of concentration upon the sorption of potassium and ammonium by the soil, three solutions of each salt were employed; namely, 0.001, 0.01, and 0.1 *N*.

It was manifestly impossible to vary the concentrations of the salts and, at the same time, to maintain constant both the ratio of salt to soil and that of soil to solution. It was therefore decided to employ a constant ratio of soil to solution and to ignore the factor of the unequal ratios of salt to soil. This appears to have been justified, since the amounts of the cations sorbed by the soils were not sufficient to decrease more than slightly the concentrations of the solutions, except in the case of the highest dilution, 0.001 *N*. Even here the maximum decrease was less than 10 per cent. Moreover, this represents an average decrease, whereas the actual decrease was probably greater than this value at the beginning of the percolation and very much less at the end.

#### SORPTION OF POTASSIUM

Fixation of potassium in nonreplaceable forms has been shown by Volk (8) and Lyman (5) to occur to only a slight extent in Hawaiian soils and then only as a result of repeatedly wetting and drying the soils. Hence, it is assumed that fixation of potassium in such forms did not occur under the conditions of this experiment and that all the potassium sorbed under the various treatments was subsequently replaced by ammonium acetate.

The results obtained for the sorption of potassium at various degrees of calcium saturation by the Hilo-coast soil are shown in figure 1. They indicate that increasing the amounts of exchangeable calcium in this soil increases its ability to sorb potassium. The degree to which exchangeable calcium is effective in increasing the sorption of potassium by the soil is seen, however, to be dependent upon the concentration of potassium in the solution with which the soil is leached. Thus, whereas saturating the completely electro-dialyzed soil with calcium approximately doubled the quantity of potassium sorbed from the 0.001 *N* KCl solution, it increased by a factor of five the amount sorbed from the 0.1 *N* solution. The slopes of the curves in the figure indicate that the beneficial effect upon the sorption of potassium of increasing amounts of exchangeable calcium is greater at the lower degrees of base saturation. This suggests that liming would prove most effective upon those soils which are most nearly devoid of exchangeable bases.

Table 2 shows results for the sorption of potassium by the Aiea soil at three stages of saturation, together with corresponding data for the Hilo-coast soil. It will be seen from this presentation that augmenting the supply of exchangeable calcium in the Aiea soil resulted, as in the case of the Hilo-coast soil, in increased sorption of potassium. In fact, the quantities of the salt sorbed by these two very diverse types of soil are, except at the highest concentration of potassium chloride, of much the same order. With the highest concentration, and at the higher degrees of calcium saturation, sorption by the Hilo-coast soil was much greater. The difference in sorption power at this point is probably conditioned in part by the very unequal exchange capacities of the two soils. Thus the amount of potassium taken up from 0.1 *N* KCl by the saturated Hilo-coast soil (17.5 m.e.) is the equivalent of the entire exchange

capacity of the Aiea soil. In every case the degree of potassium saturation resulting from the various treatments was greater for the Aiea soil.

Since potassium (from potassium chloride) apparently replaces exchangeable calcium in the soil more readily than it does exchangeable hydrogen, there seems little doubt but that increasing the degree of base saturation in the more highly leached soils of the high rainfall districts would aid substantially in bringing about a greater sorption of potassium. There is a vast difference,

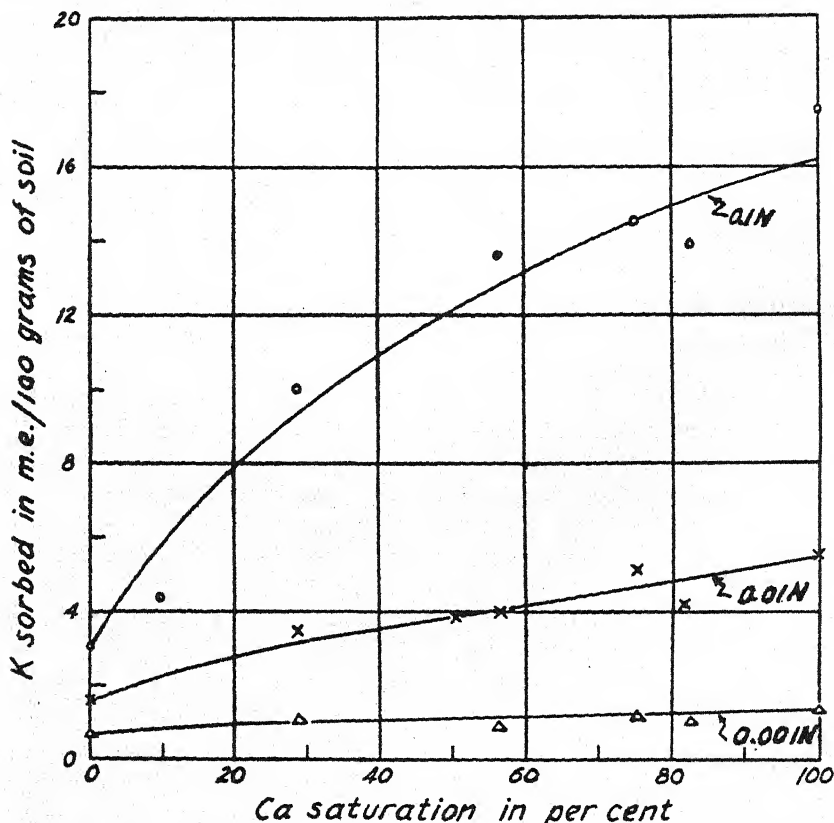


FIG. 1. SORPTION OF POTASSIUM (FROM KCl) BY THE HILO-COAST SOIL AS INFLUENCED BY CONCENTRATION AND BY THE DEGREE OF BASE SATURATION

however, between raising the level of exchangeable calcium throughout the root zone and the mere application of lime to the soil. Brown and Munsell (1) found that, after application of lime to grassland soils, a period of 10 years was required before a uniform pH was attained in the top 6 inches of soil. The problem of obtaining the necessary distribution of lime in the root zone of sugar cane would be especially difficult under Hawaiian conditions where the fields are plowed and planted only at intervals of 5 to 10 years. Hence,

it appears that, though ultimately the use of lime would prove beneficial in retarding possible losses of potassium in the high rainfall regions of the islands, little immediate gain could be expected from its use.

Figure 1 and table 2 show that the effect of the concentration of the potassium solution upon sorption of this cation by the soil is just as important as that of the degree of base saturation, if not more so. From 0.001 *N* KCl the quantities of the cation taken up were only one-fourth to one-twelfth the amounts sorbed at the same degrees of saturation from 0.1 *N* KCl. The marked decreases in the sorption of potassium with decreasing concentrations of potassium chloride, together with the relatively slight sorption of the cation from the lowest concentration (in the neighborhood of 1 m.e. for both soils, at all degrees of saturation), suggest the possibility of a concentration so low

TABLE 2

*Effect of the concentration of the percolating solution and of the degree of base saturation upon sorption of potassium and ammonium*

PERCOLATING SOLUTION	HILO-COAST SOIL			AIEA SOIL		
	Degree of Ca-saturation					
	0	51	100	0	51	100
<i>Potassium sorbed, in m.e./100 gm. of soil</i>						
0.001 N KCl.....	0.7	1.1*	1.4	0.9	...	1.2
0.01 N KCl.....	1.6	3.9	5.5	1.9	4.4	4.4
0.1 N KCl.....	3.0	12.6*	17.5	4.2	5.9	7.9
<i>Ammonium sorbed, in m.e./100 gm. of soil</i>						
0.001 N (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2	0.4	0.6	1.0	1.1	1.2
0.01 N (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.1	6.9	7.1	3.9	5.8	6.1
0.1 N (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.4	22.8	24.1	7.3	10.5	10.4

\* Obtained by interpolation from figure 1.

that no sorption whatever by the soil would occur, regardless of the degree of base saturation. Hance and co-workers (3) studying the effect upon certain Hawaiian soils of irrigation water with naturally occurring potassium to the extent of 25 p.p.m. K<sub>2</sub>O, concluded that sorption of potassium from this medium by the soil does not occur even when several hundred pounds of K<sub>2</sub>O per acre is applied annually.<sup>3</sup> Expressed on the basis of normality, this concentration of potassium corresponds to approximately 0.0005 *N*, or but one-half of the minimum concentration of potassium chloride employed in the present study, which resulted in a maximum sorption of only 1.4 m.e. of potassium per 100 gm. of soil. There seems good reason to believe, therefore, that the failure of potassium-containing irrigation water to increase the level

<sup>3</sup> It was shown, however, that the crop was able to obtain potassium directly from the irrigation water.



of exchangeable potassium in Hawaiian soils is due in very large part to the low concentration of the cation. Kelly, Brown, and Liebig, Jr. (4) and Fraps and Fudge (2) have shown that the degree to which sodium is sorbed by soils from irrigation water is dependent to a great extent upon the concentration of the cation in the medium. Their work indicates that it is due to the high dilution of the sodium in irrigation water, that the base does not normally accumulate in the soil in injurious amounts. The same workers have also shown that other cations in the irrigation water, especially calcium, influence the sorption of monovalent bases.

The repressing effect of dilution upon the sorption of potassium by the soil suggests that the application of potassium fertilizers to crops through the medium of the irrigation water may result in losses of the nutrient. The extent to which such losses of potassium might be expected to occur would depend in part upon the quantity applied and the volume of irrigation water in which the salt is dissolved. Where potassium salts are applied to the soil in crystalline forms and brought into solution through subsequent rainfall, the concentrations of the salt and hence the associated sorption of potassium would be expected to range from very high to probably negligible values.

#### SORPTION OF AMMONIUM

Data relative to sorption of ammonium (from ammonium sulfate) by the two soils at three stages of base saturation are also shown in table 2. Sorption of ammonium by both soils increased, as did that of potassium, with increasing degrees of calcium saturation, the effect being more pronounced on the Hilo-coast soil. Sorption of ammonium by the two soils was similar in amount except at the highest concentration of ammonium sulfate, 0.1 *N*. Here the effect of exchange capacity upon the sorption of ammonium is seen. Thus with the half and the completely calcium-saturated soils, sorption by the Hilo-coast soil considerably exceeded the entire exchange capacity of the Aiea soil.

Though the presence in the soil of exchangeable bases is thus seen to have a beneficial effect upon the retention of ammonium, yet a far more important factor appears to be the concentration at which the ammonium salt percolates through the soil. For example, on the Aiea soil, the quantities of ammonium sorbed from 0.1 *N*  $(\text{NH}_4)_2\text{SO}_4$  were from 8 to 10 times the amounts taken up from 0.001 *N*  $(\text{NH}_4)_2\text{SO}_4$ , whereas the maximum increases in sorption by this soil, due to base saturation, were less than twofold. The influence of concentration upon the sorption of ammonium by the Hilo-coast soil was still greater.

The results of this study suggest that when ammonium salts are applied to the soil through the medium of the irrigation water, the resulting state of sorbability of the ammonium ions is probably very low, depending upon the amount of fertilizer applied, the volume and quality of the irrigation water, and the nature of the soil. The more dilute is the solution, the greater will be the tendency for the ammonium to go where the water goes. If the water containing the salt does not penetrate the soil to depths exceeding that of the root zone, it perhaps makes little difference whether the cations are actually

sorbed by the soil or not; however, an irrigation of 7 acre-inches, which is normal under Hawaiian conditions, does in many soils reach depths considerably greater than those attained by important fractions of the sugar cane roots. Moreover, in the generally pervious soils of Hawaii, percolation of water is a fairly rapid process. Under such circumstances, it seems probable that some ammonium may be lost.

#### EFFECT OF THE ANION ON THE SORPTION OF POTASSIUM AND AMMONIUM

The results of this study showed that, at the higher concentrations of the salts, the amounts of ammonium sorbed were much greater than the corresponding quantities of potassium sorbed, particularly by the Hilo-coast soil. The question naturally arose, therefore, whether the greater sorption of ammonium at these concentrations indicated a higher sorbability of ammonium, *per se*, or whether the difference in sorption of the two cations was attributable to the different natures of the accompanying anions. It was suggested that,

TABLE 3

*Influence of the anion on the sorption of ammonium and potassium by the H-saturated Hilo-coast soil*

Results expressed in m.e./100 gm. of soil

CONCENTRATION OF SALT	POTASSIUM SORBED FROM		AMMONIUM SORBED FROM	
	KCl	K <sub>2</sub> SO <sub>4</sub>	NH <sub>4</sub> Cl	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>
<i>N</i>				
0.001	0.7	1.2	0.0	0.2
0.01	1.6	3.4	0.8	3.1
0.1	3.0	10.6	3.3	10.4

since sulfuric acid is weaker than hydrochloric acid, more ammonium than potassium was sorbed by the soils because of the union of the sulfate radical with exchangeable hydrogen to form the bisulfate radical, thereby promoting the replacement of hydrogen by ammonium.<sup>4</sup> If the respective anions were responsible for the differential sorption, then, if the soil were leached with potassium sulfate and ammonium chloride instead of with potassium chloride and ammonium sulfate, the sorption of potassium should exceed that of ammonium.<sup>5</sup> In order to test this hypothesis, portions of the completely unsaturated Hilo-coast soil were leached with solutions of potassium sulfate and ammonium chloride in the manner already described, and the extent of the resulting sorption was determined.

It will be seen from table 3 that interchanging the anions reversed the order of the sorption of ammonium and potassium: under these conditions, much more potassium than ammonium was taken up by the soil at all concentra-

<sup>4</sup> The dissociation constant for the second hydrogen of H<sub>2</sub>SO<sub>4</sub> =  $2 \times 10^{-2}$ .

<sup>5</sup> On this basis it is probable that substantial differences in the sorption of the cations from the two salts would result only where the sorption was brought about by percolation.

tions. Thus there remains no evidence that one of the two cations is more strongly sorbed than the other, when both are employed as salts of the same acid. If the proffered explanation for the observed differences in sorbability of the cations from the chloride and sulfate forms is correct, it would be expected that such differences would be greatest on the hydrogen-saturated soils and would decrease as the proportions of exchangeable hydrogen to base in the soil exchange material decreased. Such relationships appear to be implied by the data in table 2. Except at the lowest concentrations, the ratios of ammonium sorbed (from ammonium sulfate) to potassium sorbed (from potassium chloride) generally decreased with increasing degrees of base saturation. Though the evidence resulting from this test is definitely limited, the indications are that, on highly acid soils at least, greater sorption of potassium and ammonium may be expected to result from the use of sulfates than from the use of the corresponding chlorides.

#### SUMMARY

A study was made of the sorption of potassium and ammonium from 0.1, 0.01, and 0.001 *N* solutions of the cations by two Hawaiian clay soils at degrees of calcium saturation ranging from 0 to 100 per cent. The results may be summarized as follows:

Sorption of potassium and ammonium (from percolating solutions) decreased greatly with decreases in concentrations of the cations. At the lowest concentration (0.001*N*) the sorption was very low, ranging from 0 to 1.4 m.e. per 100 gm. of soil, depending upon the salt used and the degree of calcium saturation.

Increasing the degree of calcium saturation of the soils increased the sorption of potassium and ammonium. The effect was not so marked, however, as was that of the concentration.

Sorption of potassium and ammonium (by the completely electrodyalyzed soil) was much higher from the sulfates than from the chlorides of these cations.

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# A COMPARISON OF THE BRIGGS-McLANE AND THE GOLDBECK-JACKSON CENTRIFUGE METHODS FOR DETERMINING THE MOISTURE EQUIVALENT OF SOILS<sup>1</sup>

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The moisture equivalent was first proposed by Briggs and McLane (4), of the Bureau of Soils, in 1907. Since then the equipment and the procedure for determining it have been refined until now it is generally accepted by soil scientists as one of the important physical measurements of soil. In 1929 Bouyoucos (1) proposed a suction method for estimating the moisture equivalent and compared it with the centrifuge method. His data in general show reasonably good agreement between the two methods (2). Recently Pinckney and Alway (6) made a comparison of the centrifuge and suction methods with 113 soils from Minnesota and concluded that "though the relationships are in general agreement with those reported by Bouyoucos, they do not fully support his conclusion as to reliability and desirability of the method" and that "only where the moisture equivalent centrifuge is not available does the use of the suction method appear desirable, and the value so obtained should be referred to by some other designation than moisture equivalent."

Goldbeck and Jackson (5), of the Bureau of Public Roads, proposed a method for determining the moisture equivalent of clay material for road work by using a regular centrifuge equipped with Babcock trunnion cups and with Gooch crucibles as containers for the soil. Their method has been modified until now (3, 9) it differs from the Briggs and McLane method only in the type of equipment, the method of preparing the samples for analysis, and the time necessary for centrifuging. The moisture-equivalent centrifuge is expensive and is not available in all soil laboratories, but most laboratories have a regular centrifuge equipped with trunnion cups and rheostatic speed control. If the two methods give comparable results, the procedure whereby the determinations are carried out in Gooch crucibles using a regular centrifuge would be of value, therefore, to workers not having access to a moisture-equivalent centrifuge.

<sup>1</sup> Cooperative investigations of the office of research, Soil Conservation Service, U. S. Department of Agriculture, and the West Virginia Agricultural Experiment Station. Published with the approval of the director, W. Va. Agricultural Experiment Station, as Scientific Paper No. 251.

<sup>2</sup> Soil Conservationist, U. S. Department of Agriculture.

<sup>3</sup> Cooperative Agent, U. S. Department of Agriculture and the W. Va. Agricultural Experiment Station.

While methods for determining pore-size distribution in soils were being studied, undisturbed and disturbed samples were subjected to different centrifugal forces in specially constructed brass tubes and in Gooch crucibles, respectively. Moisture equivalents by the standard moisture-equivalent centrifuge were also available on these soils. The data reported herein are a comparison of the moisture equivalents obtained by the Briggs-McLane and the Jackson-Goldbeck procedures on 58 soil samples that differ widely in their physical and chemical properties.

#### PROCEDURE

The soil samples were prepared for analysis by being screened, while air-dry, through a 2.0-mm. sieve. The Briggs-McLane centrifuge<sup>4</sup> was used for determining the moisture equivalent in the usual manner.

A regular SB-1 International centrifuge, equipped with ordinary rheostatic speed control and Babcock trunnion cups,<sup>5</sup> with Gooch crucibles as containers for the soil, was used for determining the moisture equivalent as proposed by Goldbeck and Jackson (5). Into each Gooch crucible, fitted with filter paper, 5 gm. of soil was weighed, after which the procedure for saturating, draining, and centrifuging the sample was identical with that followed in determining the moisture equivalent by the standard method. In this study the desired speed was controlled to within about 50 r.p.m. with an International centrifuge tachometer, No. 748.

#### RESULTS

The soil number, soil type, location, moisture equivalents determined by the two methods, and the difference between the two determinations are shown in table 1. It is to be observed that the soils differ widely in origin and texture. The range of moisture equivalent, determined in the Briggs and McLane centrifuge, is from 3.9 to 40.9. In column 6 are shown the differences between the two methods, the results obtained by the standard moisture-equivalent centrifuge being used as a basis of comparison. The general tendency is for the readings to be lower when determinations are made in Gooch crucibles. The range of differences in the moisture content between the two methods is from -0.9 to +1.9, inclusive, with 37 of the samples varying less than 1 per cent. In only 5 of the 58 samples do the values in the Gooch crucibles exceed those obtained by the Briggs-McLane method. The 58 determinations made in the Gooch crucibles on the average contained 0.76 per cent less moisture than those made in the standard moisture-equivalent centrifuge. The standard error being .075, the difference between methods is highly significant. The reasons for these consistently low results are not readily apparent, since the centrifugal

<sup>4</sup> The authors are indebted to T. C. Peele, Soil Conservation Service, Clemson, South Carolina, for these moisture-equivalent determinations.

<sup>5</sup> If a centrifuge is available, the necessary equipment for this determination may be obtained from the Central Scientific Co. at an approximate cost of \$15.

TABLE 1

*Moisture equivalents determined by the Briggs-McLane and the Goldbeck-Jackson centrifuge methods*

SOIL NUMBER	SOIL TYPE	LOCATION	MOISTURE EQUIVALENT		
			By standard procedure	By Gooch crucibles	Difference
32A*	Ruston sandy loam	Georgia	3.9	2.7	1.2
23A	Ruston sandy loam	Georgia	4.3	2.9	1.4
12A	Ruston sandy loam	Georgia	4.9	3.0	1.9
129A	Vernon fine sandy loam	Oklahoma	7.6	6.8	0.8
12B	Ruston sandy loam	Georgia	8.7	7.2	1.5
32B	Ruston sandy loam	Georgia	9.0	7.7	1.3
129B	Vernon fine sandy loam	New York	11.1	10.2	0.9
34A	Red Bay loam	Georgia	11.7	10.3	1.4
125A	Kirkland sandy clay	Oklahoma	13.8	12.8	1.0
128A	Parsons fine sandy loam	Oklahoma	14.7	14.6	0.1
117B	Vernon fine sandy loam	Oklahoma	14.9	13.1	1.8
34B	Red Bay loam	Georgia	15.3	14.0	1.3
135B	Hopi sandy loam	New Mexico	15.8	14.6	1.2
11A	Greenville sandy clay loam	Georgia	16.1	14.5	1.6
134A	Pinedale clay loam	New Mexico	17.3	17.1	0.2
141A	Melbourne loam	Oregon	17.7	16.7	1.0
126B	Kirkland fine sandy loam	Oklahoma	18.4	17.2	1.2
22B	Honeoye gravelly silt loam	New York	18.6	18.3	0.3
20B	Bath gravelly silt loam	New York	19.3	18.6	0.7
127A	Bates very fine sandy loam	Oklahoma	19.7	18.8	0.9
16B	Honeoye gravelly silt loam	New York	19.9	19.3	0.6
16A	Honeoye gravelly silt loam	New York	20.0	19.5	0.5
110A	Muskingum silt loam	Ohio	21.4	21.2	0.2
143B	Walla Walla silt loam	Washington	20.9	20.1	0.8
115A	Miami silty clay loam	Ohio	21.3	20.3	1.0
22A	Honeoye gravelly silt loam	New York	22.2	21.8	0.4
11B	Greenville sandy clay loam	Georgia	22.6	22.0	0.6
10A	Hagerstown clay loam	West Virginia	22.7	21.9	0.8
109A	Muskingum silt loam	Ohio	22.7	21.7	1.0
6B	Dekalb silt loam	West Virginia	23.2	21.8	1.4
2B	Hagerstown clay loam	West Virginia	23.2	22.3	0.9
115B	Miami silty clay loam	Ohio	23.2	22.2	1.0
1A	Dekalb silt loam	West Virginia	23.5	22.4	1.1
111A	Muskingum silt loam	Ohio	23.5	22.6	0.9
23A	Honeoye gravelly silt loam	New York	23.5	23.1	0.4
20A	Bath gravelly silt loam	New York	24.8	24.1	0.7
146A	Palouse silt loam	Washington	23.6	22.7	0.9
9A	Susquehanna clay loam	Georgia	24.0	24.3	-0.3
118B	Austin clay	Texas	24.2	23.5	0.7
106B	Fayette silt loam	Minnesota	24.3	23.0	1.3
33A	Orangeburg sandy loam	Georgia	24.5	22.9	1.6
11B	Greenville sandy clay loam	Georgia	22.6	22.0	0.6

\* A and B refer to surface and subsurface soils, respectively.

TABLE 1—*Concluded*

SOIL NUMBER	SOIL TYPE	LOCATION	MOISTURE EQUIVALENT		
			By standard procedure	By Gooch crucibles	Difference
17A	Honeoye gravelly silt loam	New York	25.0	24.6	0.4
140B	Aiken clay loam	Oregon	25.0	24.2	0.8
113A	Upshur clay	Ohio	25.1	25.1	0.0
112A	Westmoreland clayey silt loam	Ohio	26.2	25.8	0.4
17B	Upshur clay	West Virginia	25.7	25.2	0.5
21A	Fremont gravelly silt loam	New York	26.8	27.3	-0.5
114A	Muskingum silt loam	Ohio	26.9	26.2	0.7
14A	Upshur clay	West Virginia	27.0	27.9	-0.9
15A	Monongahela silt loam	West Virginia	26.0	25.4	0.6
119A	Austin clay	Texas	26.7	26.6	0.1
105A	Carrington silt loam	Minnesota	26.9	25.6	1.3
112B	Westmoreland clayey silt loam	Ohio	27.9	27.7	0.2
18A	Honeoye gravelly silt loam	New York	30.4	30.5	-0.1
122A	Houston clay	Texas	33.0	33.1	-0.1
124B	Houston clay	Texas	33.9	33.4	0.5
9B	Susquehanna clay loam	Georgia	40.9	40.1	0.8

force is the same in both methods. Thickness of the soil samples has been shown (8) to influence the moisture equivalent, and the slightly lower results obtained in the Gooch crucibles may be explained partly on this basis. The design of the equipment might suggest greater tendency for evaporation during centrifuging, but samples centrifuged in specially constructed air-tight brass tubes gave the same results as Gooch crucibles, indicating that evaporation is not a factor.

Since the values obtained in Gooch crucibles are in general low, the possibility of decreasing the centrifugal force to obtain values more in line with the standard moisture equivalent was tried but without success. It was found that decreasing the centrifugal force had a much smaller effect on the moisture content of the coarse-textured soils than on that of the fine-textured soils. For example, the moisture content of a coarse-textured soil was increased from 3.0 to 3.6 by decreasing the centrifugal force from 1000 to 750. The same change in centrifugal force increased the moisture content of a fine-textured soil from 40.1 to 42.9. The change in moisture content was 0.6 per cent in the former, and 2.8 per cent in the latter. The moisture equivalent by the standard procedure was 4.9 for the coarse-textured soil and 40.9 for the fine-textured soil; consequently the values for the former are 1.3 low, whereas those for the latter are 2.8 high. It is also evident from table 1 that the differences between the two methods are, on the average, greater for the soils with low moisture equivalents.

Duplicate determinations in the Briggs-McLane machine in general were somewhat closer than those carried out in Gooch crucibles. The mean differ-

ence between duplicate determinations made by the Briggs-McLane procedure was  $.188 \pm .033$ , whereas that between the duplicate determinations made in the Gooch crucible was  $.414 \pm .058$ . If a closer speed control had been used, as described by Russell and Richards (7), duplication in Gooch crucibles probably would be as close as in the Briggs-McLane machine.

Estimates of standard-procedure values can be obtained from Gooch crucible values by means of the following equation:

$$S = 1.60 + .96 G$$

in which  $S$  = standard-method value in per cent

$G$  = Gooch crucible method value in per cent.

Although, if a correction is not used, the percentage error between the two methods is large for soils with low moisture equivalent, the absolute difference in moisture does not vary widely over the entire range of moisture equivalent studied.

From a review of the investigations on factors affecting the moisture-equivalent determination with the Briggs-McLane centrifuge, it is evident that differences of 0.5 or more are often obtained by different investigators or even by two individuals working in the same laboratory, depending largely upon the method of preparing the sample for analysis. For practical purposes a difference of 1 per cent in moisture is probably satisfactory. Therefore, when a standard moisture-equivalent machine is not available, a regular centrifuge equipped with trunnion cups can be used for determining the moisture equivalent.

#### SUMMARY

Comparison was made of the moisture equivalents for 58 samples of a number of important soil types of the United States determined in a regular centrifuge equipped with trunnion cups and with Gooch crucibles as containers for the soil, as described by Goldbeck and Jackson, and in the Briggs-McLane centrifuge.

The moisture equivalents by the Briggs-McLane procedure ranged from 3.9 to 40.9 inclusive. The range in difference between the methods was  $-0.9$  to  $+1.9$  inclusive; the Gooch crucible procedure averaging 0.76 per cent less than determinations made in the Briggs-McLane moisture-equivalent machine. In this study a significant difference existed between the two methods unless all values obtained in Gooch crucibles were corrected by use of the regression equation between the two methods.

When a Briggs-McLane moisture-equivalent centrifuge is not available, results satisfactory for most purposes can be obtained by using the equipment recommended by Goldbeck and Jackson.

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## A RAPID METHOD FOR DETERMINING SOIL MOISTURE

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The method described in this paper is valuable for the quick determination of the percentage of moisture in soil, especially when many determinations are to be made upon the same kind of soil. The method is also applicable to other materials, such as green plants, grains, sand, and gravel. The only pieces of apparatus required are some flasks and a balance, preferably automatic, weighing about 200 gm. to an accuracy of 0.1 gm. Only two weighings are necessary.

The procedure is as follows: Place 50 gm. of soil in a flask bearing a volume mark at about 100 cc. Add tap water, shaking at the same time in order to eliminate soil air. Make up to the volume, and weigh. Subtract from this weight that of the flask made up to the same volume with only water. The difference multiplied by a factor gives the oven-dry weight of the soil. The factor is determined once for each kind of soil by oven drying a sample and dividing the oven-dry weight by the aforementioned difference.

The factor varied from 1.563 to 1.667 for 17 soils tested; it is about 2.80 for wheat grains, 3 for maize grains, 2.73 for green wheat plants, 1.623 for kaolin, 1.639 for chalk, 1.612 for sand, and 1.618 for gravel.

In order to eliminate the air readily, it is best to put a little water into the flask first, then add the material, and finally make up to the volume. To avoid frothing, the flask must be heated; an air pump may be used for extracting the air, and air bubbles may also be dispersed with a glass rod. For ordinary determinations of soil moisture, however, these precautions are not necessary. Table 1 gives a comparison of the oven-dry weights determined by actual drying and those calculated by the rapid method applied without the special precautions for eliminating air.

The quotient 
$$\frac{\text{weight after ignition} + \text{CO}_2 \text{ of carbonates}}{\text{difference in weight of the flasks}}$$
 was also calculated.

This quotient is more constant than the factor for oven-dry weight (see table 1). It varied from 1.461 to 1.5017 for 17 soils<sup>1</sup> tested; for gravel and for soils resembling gravel it is higher. The lower specific gravity of gravel may be attributed to air-filled interstices in the interior.

<sup>1</sup> Table 1 gives the results of only a few of the soils tested.

TABLE 1

*Results of the rapid method for determining soil moisture*  
 (Figures correspond to unity as the weight of moist soil)

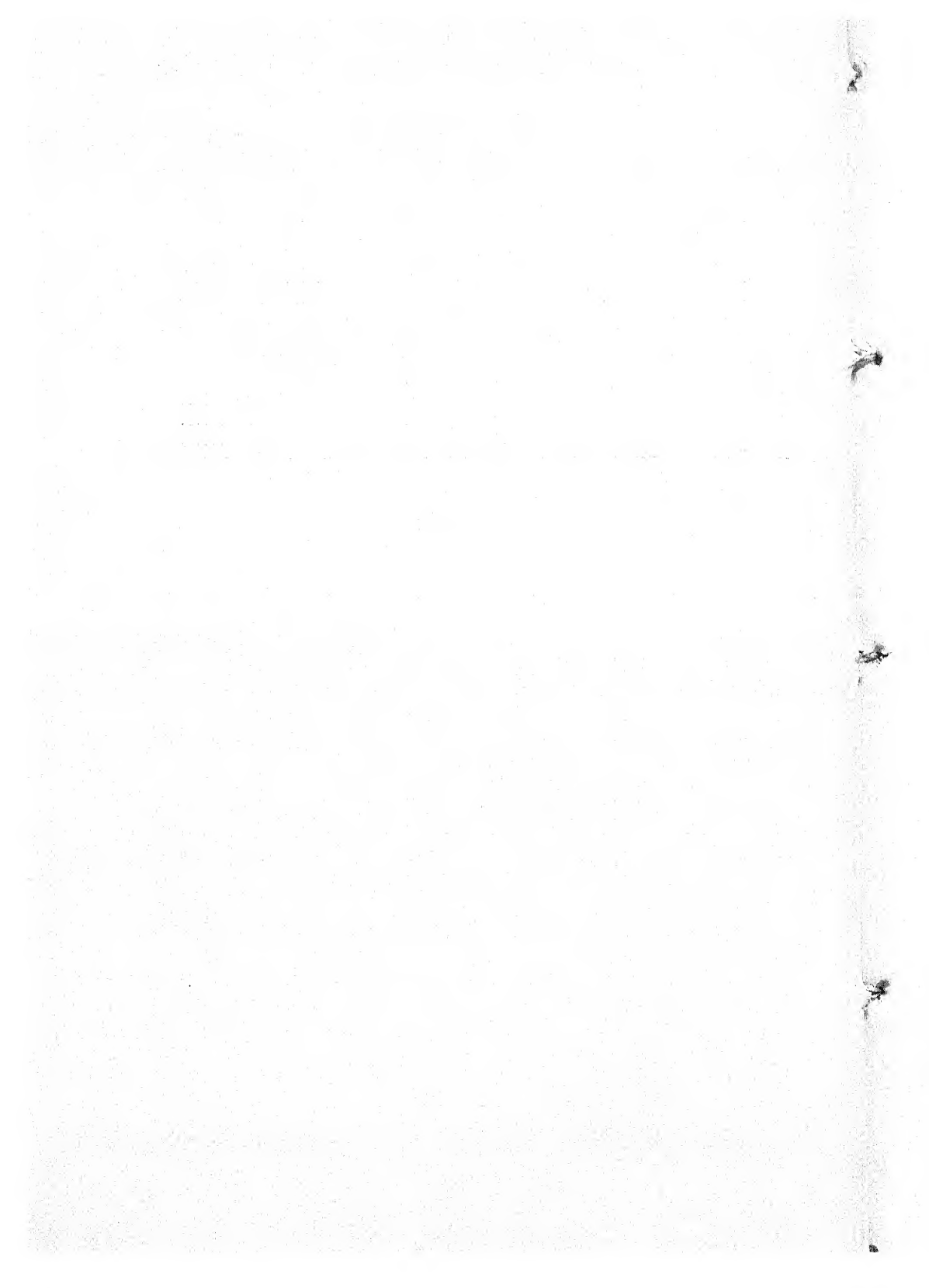
NUMBER	SOIL	OVEN-DRY WEIGHT		WEIGHT AFTER IGNITION	
		Actual*	Calculated	Actual*	Calculated
1	Alluvial very sandy soil of Inst. dry exp. field	0.9489	0.9503	0.8953	0.9001
2	Alluvial sandy soil	0.9019	0.8909	0.8483	0.8439
3		0.9185	0.9193	0.8649	0.8708
4		0.9279	0.9252	0.8743	0.8764
5		0.8894	0.8893	0.8358	0.8424
6	Alluvial very sandy soil	0.9184	0.9109	0.8648	0.8628
7	Alluvial sandy soil	0.9554	0.9484	0.9018	0.8984
8		0.9159	0.9278	0.8623	0.8788
9		0.9159	0.9187	0.8623	0.8702
10		0.9159	0.9222	0.8623	0.8735
11		0.9683	0.9778	0.9147	0.9262
12		0.9683	0.9628	0.9147	0.9198
13		0.9683	0.9678	0.9147	0.9167
14	Red soil from Si rock, Inst. Thermi exp. field	0.8240	0.8247	0.7618	0.7766
15		0.9040	0.8938	0.8418	0.8418
16		0.9232	0.9300	0.8610	0.8759
17		0.8905	0.8835	0.8283	0.8320
18		0.8905	0.8835	0.8283	0.8320
19		0.8905	0.8973	0.8283	0.8476
20		0.8627	0.8680	0.8005	0.8175
21		0.8627	0.8630	0.8005	0.8128
22		0.8627	0.8614	0.8005	0.8113
23		0.9352	0.9382	0.8730	0.8836
24	Terra rossa of St.Paraskevi, Thessaloniki	0.9178	0.9177	0.8193	0.8234
25		0.9178	0.9138	0.8193	0.8199
26		0.9178	0.9171	0.8193	0.8229
27		0.9430	0.9484	0.8445	0.8510
28		0.9430	0.9395	0.8445	0.8430
29		0.9430	0.9448	0.8445	0.8478
30	Redzina soil of N.Plaghari†	0.8438	0.8437	.....	0.7710
31	Humus-rich soil of Inst.	0.8718	0.8718	.....	0.8057
32		0.8238	0.8209	0.7451	0.7545
33		0.8238	0.8306	0.7451	0.7633
34		0.8238	0.8193	0.7451	0.7530
35		0.8690	0.8725	.....	0.7749

\* Determined by the usual method.

† Only one determination.

TABLE 1—*Concluded*

NUM- BER	SOIL	OVEN-DRY WEIGHT		WEIGHT AFTER IGNITION	
		Actual*	Calculated	Actual*	Calculated
36	Humus-rich soil of Inst.—	0.8690	0.8755	.....	0.7776
37	<i>Continued</i>	0.8690	0.8695	.....	0.7722
38		0.8690	0.8682	.....	0.7710
39		0.8690	0.8585	.....	0.7625
40		0.6277	0.6274	.....	0.5573
41	Gravel of Epanomi	0.9977	0.9926	0.9914	.....
42		0.9977	1.0007	0.9914	.....
43		0.9977	0.9968	0.9914	.....
44		0.9951	0.9932	0.9888	.....
45		0.9951	0.9942	0.9888	.....
46		0.9951	0.9990	0.9888	.....



## THE USE OF RADIOACTIVE ELEMENTS FOR SOIL AND FERTILIZER STUDIES

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The production of artificially radioactive elements in appreciable quantities by the cyclotron (1, 3, 4, 5) makes available a new tool for soil and fertilizer studies. Samples of a fertilizer may be synthesized from the radioactive isotope of the element, and if this fertilizer is applied to a soil it can be located subsequently with a detector for the beta and gamma rays given off by the radioactive atoms. The most suitable detecting instrument is the Geiger-Müller counter because of its small dimensions and high sensitivity.

Some preliminary experiments on the fixation of phosphorus by several soil types have been carried out with radioactive phosphorus as an indicator. This was prepared by bombarding red phosphorus with deuterons, a procedure that transforms a small fraction of the phosphorus into radioactive  $P^{32}$  which has a half life of 14.3 days. The phosphorus was then dissolved in nitric acid and the solution heated to convert the phosphorus to  $H_3PO_4$  and drive off the oxides of nitrogen. The solution was diluted and titrated to a pH of 3.0 with saturated  $Ca(OH)_2$ , at which point  $CaHPO_4$  begins to precipitate. The solution was increased to a volume of 1 liter and the phosphorus content determined by the standard method.

An aliquot of this solution was then poured on top of the soil column in a cardboard container 4 inches in diameter and 8 inches high. Distilled water was added to wet the soil to the bottom. Then the soil and the box were sliced in a vertical direction, and the distribution of the radioactive phosphorus in the vertical section was found with the Geiger-Müller counter (2). This was done by placing the section in a horizontal position under the counter, as indicated in figure 1. The number of counts recorded at any depth gives the amount of phosphorus that has penetrated to that depth. The layer of soil measured at each setting was three-eighths inches deep and the count recorded is taken to be the intensity of radioactive phosphorus at the average depth. It has subsequently turned out that there may be a large change in intensity in three-eighths inch and it would therefore be advantageous to use a thinner layer, say one-eighth inch deep. The lead shield insures that beta rays from other portions of the soil do not enter the counter. If gamma rays

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are present, corrections must be made for them. Radioactive phosphorus does not emit gamma rays, and the recorded count corrected for the natural background of the counter is due only to the portion of the soil directly under the opening in the lead shield.

The curves in figure 2 show some results obtained with this technic. Equal weights of four soils—Cecil clay, Newton sandy loam, Crosby silt loam, Bedford silt loam—were treated in exactly the same way by first adding 144 cc. of the  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  solution. The solution contained 314 mgm.  $\text{P}_2\text{O}_5$  per 144 cc., which added to 1600 gm. of soil is equivalent to about 400 pounds of  $\text{P}_2\text{O}_5$  per acre. When the solution had penetrated and disappeared, 350 cc. of water was added to wet the soil to the bottom of the container. The total water

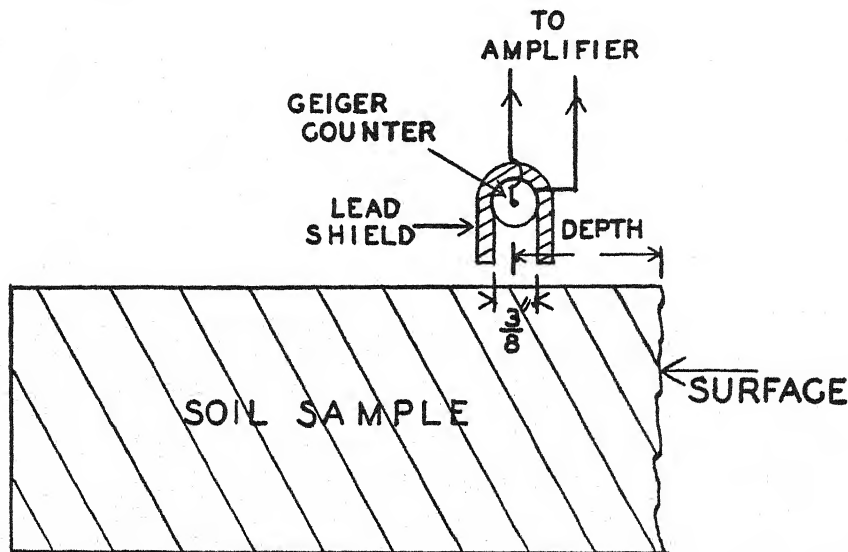


FIG. 1. EXPERIMENTAL ARRANGEMENT TO MEASURE THE DISTRIBUTION OF RADIOACTIVE PHOSPHORUS IN THE SOIL COLUMN

added corresponded to a rainfall of 2.5 inches. The samples were then sliced from top to bottom, and the distribution of the phosphorus was measured with the Geiger-Müller counter as described above. It is evident from the result that the phosphorus does not penetrate very far. In each case the majority of the phosphorus remains in the upper  $1\frac{1}{2}$  inches of the soil. The curves also indicate the relative fixing powers of the various soils for phosphorus. The curves cannot be accurately extrapolated to the surface, since the first reading was at an average depth of three-eighths inch, but still an estimate can be made of the penetration. For example, in Cecil clay, which has a high fixation for phosphorus, not more than 5 per cent of the added phosphate penetrated beyond 1 inch, and more than 50 per cent remained in the top three-eighths inch. For the other soils the penetration was greater. The

depths of the layers which fix 95 per cent and 50 per cent of the phosphorus are given in table 1.

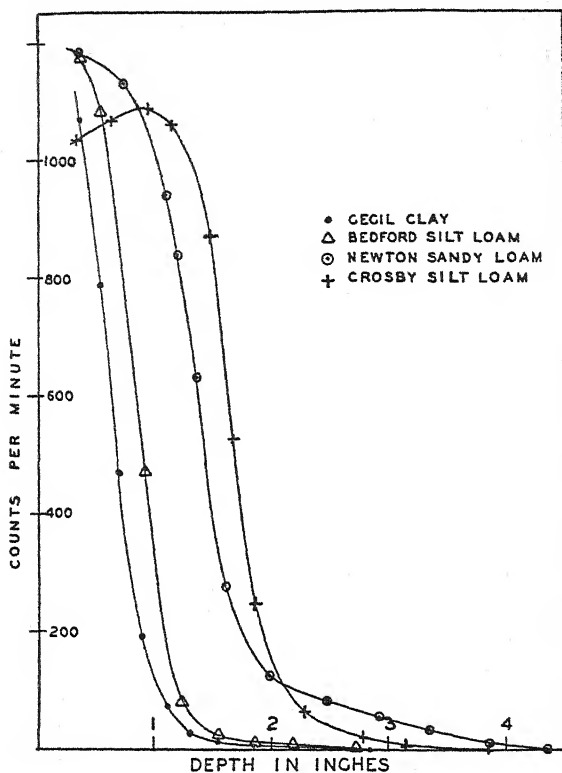


FIG. 2. FIXATION OF PHOSPHORUS BY FOUR DIFFERENT SOILS

Each soil received 144 cc.  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  solution containing radioactive phosphorus plus 350 cc. water.

TABLE 1  
*Penetration of soluble phosphate*

SOIL	95 PER CENT P REMAINS ABOVE	50 PER CENT P REMAINS ABOVE
	inches	inches
Cecil clay.....	1	$\frac{3}{8}$
Bedford silt loam.....	$1\frac{1}{16}$	$\frac{1}{2}$
Newton sandy loam.....	$1\frac{1}{8}$	$\frac{3}{4}$
Crosby silt loam.....	$1\frac{7}{8}$	$\frac{7}{8}$

The curves give only relative values and should not be compared on an absolute basis, since no particular precaution was taken to ensure the same separation from the cut surface to the counter in each case. These results



show that soluble phosphate fertilizers put on the top of these soils do not penetrate to an extent likely to reach the roots of plants feeding deeply in the soil.

The effect of KCl and  $(\text{NH}_4)_2\text{SO}_4$  on the fixation of phosphorus by the Bedford soil was examined. Three similar samples were treated as above with 100 cc. solution containing radioactive phosphorus. After this solution was ab-

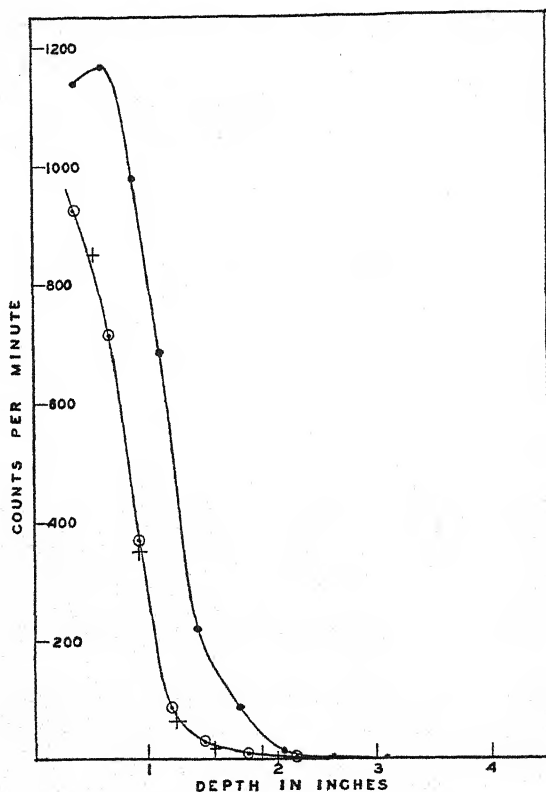


FIG. 3. EFFECT OF KCl AND  $(\text{NH}_4)_2\text{SO}_4$  ON THE FIXATION OF PHOSPHORUS IN BEDFORD SILT LOAM

+ = radioactive phosphorus solution plus 300 cc. water; ⊙ = radioactive phosphorus solution plus 300 cc. water in which was dissolved 4.8 gm.  $(\text{NH}_4)_2\text{SO}_4$ ; ● = radioactive phosphorus solution plus 300 cc. water in which was dissolved 4.8 gm. KCl.

sorbed, 350 cc. distilled water was added to one of the samples and to the others 350 cc. water in which was dissolved 4.8 gm. of inactive KCl and  $(\text{NH}_4)_2\text{SO}_4$  respectively. The resulting distribution of radioactive phosphorus is shown in figure 3. The  $(\text{NH}_4)_2\text{SO}_4$  had no direct effect on the distribution, and the KCl apparently caused the phosphate to move down more than where no KCl was used. The rising portion of the curve in the region of one-half inch sug-

gests that the phosphorus has been partly removed from the top layers of the soil by the KCl treatment.

One experiment was tried on the adsorption of potassium, using radioactive potassium as an indicator. Potassium chloride was bombarded with deuterons to produce  $K^{38}$  with a half life of 12.4 hours. The KCl was dissolved in water, and 200 cc. of the solution containing 500 mgm. KCl was poured on a sample of Bedford silt loam. The soil was then wet down with 300 cc. of water. The KCl added was equivalent to 600 pounds per acre and the water to a precipitation of 2.5 inches. The distribution of the radioactive potassium

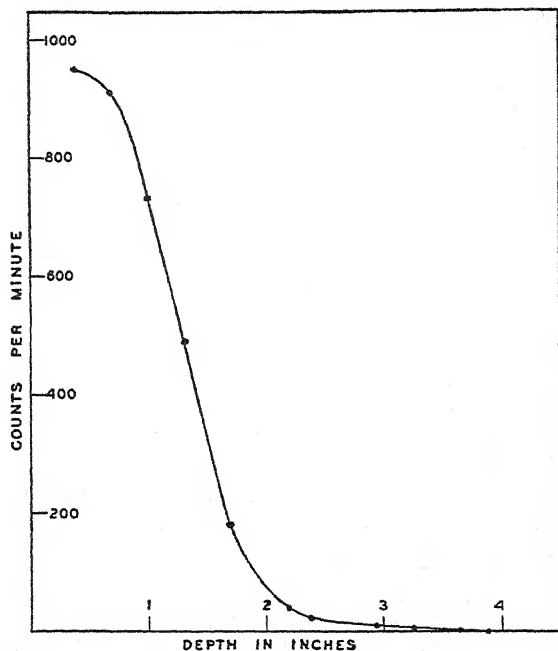


FIG. 4. ADSORPTION OF POTASSIUM BY BEDFORD SILT LOAM

To the soil was added 100 cc. solution containing radioactive KCl plus 350 cc. water.

was measured in the same manner as the phosphorus. The results, which are plotted in figure 4, show that 95 per cent of the potassium chloride applied in this concentration to this soil did not penetrate beyond  $1\frac{5}{8}$  inches.

#### SUMMARY

The fixation of phosphorus by Cecil clay, Bedford silt loam, Newton sandy loam, and Crosby silt loam has been investigated by the use of the radioactive isotope. When  $Ca(H_2PO_4)_2$  containing radioactivated phosphorus was applied to the surface of the soil and washed down with water equivalent to a precipitation of 2.5 inches the penetration of the phosphorus ranged from

1½ inches for Cecil clay to about 4 inches for Crosby silt loam. The addition of KCl caused the radioactive phosphorus to move down into the soil more than where no KCl was used, but  $(\text{NH}_4)_2\text{SO}_4$  had no immediate effect.

The radioactive technic was used to study the movement of potassium in Bedford silt loam. It was found that not more than 5 per cent of the KCl applied penetrated beyond 1½ inches with a water treatment equivalent to 2.5 inches of rainfall.

The radioactive technic appears to present a valuable method for studying the behavior of ions in soils.

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# METHODS FOR DETERMINING PHOSPHATE IN SOIL EXTRACTS

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During the course of an investigation of certain Nyasaland soils an attempt was made to apply the method of Warren and Pugh (7) to the determination of hydrochloric acid soluble phosphoric acid, but it was found that the method failed to give satisfactory results with these soils. The work reported in this paper resulted from an attempt to overcome this difficulty.

## INTRODUCTION

### *Soils and extracts*

The soils under investigation were representative of apparently similar red earths found on the Native Tobacco Board's experimental stations at Likuni and Chimvua in the Lilongwe district, Nyasaland. Sixteen samples which were representative of the A and B horizons of eight profiles—four at Likuni and four at Chimvua—were selected for phosphate analysis. For the present purpose it will be sufficient to refer to these samples, and to the hydrochloric acid extracts derived from them, as L1 to L8 and C1 to C8 and to note that an odd suffix indicates a surface soil, and an even suffix, a B horizon. The organic carbon content of the soils, as determined by the Walkley-Black (5) method (uncorrected), ranged from 1.67 to 0.23 per cent.

The hydrochloric acid extracts were prepared by the A.E.A. method of 1905 as described by Warren and Pugh (7). In each case 20 gm. of soil was used, and the final volumes of the extracts (aliquots of which were used for analysis) were adjusted to 250 cc.

### *Colorimetric estimation of phosphate*

The Denigès method, first applied to soils by Atkins (1) and subsequently used by Parker and Fudge (3) and by Warren and Pugh (7), has been employed in all the work reported in this paper, and the molybdic acid and the stannous chloride reagents were prepared according to the directions given by Warren and Pugh. The blue phosphate color produced by these reagents appears to be more sensitive to traces of ferric iron than that produced by the Truog and Meyer reagents (4), but in the virtual absence of iron both sets of reagents were found to give identical results.

*Color development* was originally carried out on the lines advocated by Warren

and Pugh, but the following technique was finally adopted and found to give very satisfactory results:

Into a 100-cc. standard flask, 1 cc. of molybdic reagent was introduced, the appropriate volume of phosphate solution added, and the volume made up to the mark. The solution was then transferred to a 250-cc. conical flask, where color development was started, whenever convenient, by the addition of 3 drops of stannous chloride reagent.

It was considered that this method has two advantages over the original: first, the principle, originally stated by Chapman (2), for minimizing the effect of silica is employed; and, second, because of the speed with which the stannous chloride can be added to, and mixed with, the contents of the larger flasks, color development can be started in a number of flasks within a few seconds of one another. Under such circumstances, color developments and fading follow parallel courses, and consequently color comparisons may be made at any time from 5 to 30 minutes after the addition of the stannous chloride.

*Comparison* was made against standards derived from a solution of  $K_2HPO_4$  containing 0.01 mgm.  $P_2O_5$  per milliliter. It was found that 4 cc. of this solution (0.04 mgm.  $P_2O_5$ ) gave a very convenient depth of color and latterly this standard was always employed, the amount of unknown being adjusted to give an approximate match. A Hellige colorimeter with artificial illumination was used, and the unknown was first matched against the standard and then the standard against itself. This latter reading was taken for calculation in order to eliminate any variation between the two halves of the colorimeter.

### *Acidities*

The pH values of solutions were, in all cases, determined by the quinhydrone electrode with 0.01 *N* HCl: 0.09 *N* KCl as standard half cell.

### METHODS APPLIED TO THE SOIL EXTRACTS

#### *The original Warren and Pugh method*

The Warren and Pugh method was designed to remove organic matter and iron from solution prior to the estimation of phosphoric acid by the Denigès method. The following solutions, based on those described by Warren and Pugh, have been employed in all the work reported in this paper: a saturated solution of potassium permanganate, containing about 6.6 per cent  $KMnO_4$ ; a solution of potassium ferrocyanide, containing 10 per cent  $K_4Fe(CN)_6 \cdot 3H_2O$ ; a solution of manganese sulfate, containing 10 per cent  $MnSO_4 \cdot 2H_2O$ ; an approximately 6*N* ammonia solution; and a 2*N* sulfuric acid solution.

The original method was applied to the Chimvua extracts by gently heating a mixture of 15 cc. extract and 2 cc. of permanganate solution on a sand bath until the precipitate of manganese oxide completely dissolved and thereafter cooling and diluting to about 35 milliliters. Six milliliters of ferrocyanide solution, followed by 5 cc. manganese solution, were added with shaking and,

after standing for a few minutes, the solution was neutralized by ammonia until the color of the ferrocyanide precipitate changed to purple. Acidity was next restored by the addition of 3.5 cc. of sulfuric acid, the volume made up to 100 cc., and the precipitate removed by filtration. The filtrate comprising the first few milliliters was rejected, and aliquots of the main filtrate were used for color development.

The method outlined is identical with that described by Warren and Pugh except for the substitution of potassium permanganate for the 0.5 cc. of 20 per cent sodium permanganate used by them. Warren and Pugh, in discussing the method, indicate that neutralization to a pH of about 6.8 is necessary to complete precipitation and that the addition of 3.5 cc. of 2*N* sulfuric acid produces a final pH of approximately 3.0 in the solution.

The results obtained with the extracts from the Chimvua soils indicated that the estimations of phosphate were very irregular (table 1), and experiments with added phosphate showed very incomplete recoveries. Investiga-

TABLE 1  
*Phosphate in Chimvua extracts as determined by the original Warren and Pugh method*  
Mgm.  $P_2O_5$  in 15 cc. extract

SERIES	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8
1	0.103	0.111	0.135	0.092	.....	0.108	0.064	0.084
2	0.109	0.032	0.132	.....	0.067	.....	0.384	0.234
3	.....	0.127	.....	0.116	0.122	0.067	0.338	0.172

tion of the filtrates revealed incomplete removal of iron and very variable acidities, which averaged a pH of 3.7 instead of 3.0.

#### *Modifications of the Warren and Pugh method*

Attempts to overcome the difficulties of the original method merely by increasing the amounts of ferrocyanide and acid led to very greatly increased estimates of phosphate but failed to give consistent results; and it was concluded that hydrochloric acid extracts from different soils possess different buffer actions which prevent the original method of acidity adjustment from giving regular pH values in the final solution. It is noteworthy that, with the addition of 3.5 cc. of 2*N* acid, Warren and Pugh reported a final pH of 3.0, with the Nyasaland soils very variable acidities, averaging pH 3.7, were obtained, and when the same procedure was applied to a hydrochloric acid solution containing 0.5 gm. of ferric iron, a final pH of 1.45 was obtained.

Subsequent experiments indicated that

Iron and manganese ferrocyanides could be completely precipitated in comparatively acid solution (pH 1.0 to 2.0) and at such acidities no phosphate was coprecipitated. The neutralization to a pH of 6.8 and subsequent reacidification thus appeared unnecessary, and it was concluded that a pH of 3.0 was not acid enough in the case of the extracts from the Nyasaland soils.

The necessary adjustment of acidity, prior to precipitation, could be made by taking advantage of the change in color from yellow to orange which occurs when solutions of ferric iron are neutralized to a point just before a permanent precipitate forms. The addition of a definite amount of standard acid to the orange solution obtained by neutralizing the soil extracts with ammonia was found to give a final pH which was largely independent of the soil extract employed.

The amount of ferrocyanide was not critical, provided sufficient was added to remove all the iron.

The amount of manganese sulfate was not critical, provided sufficient was added to remove all excess ferrocyanide.

In the case of these soils, the organic matter was not sufficient to interfere with the method, since the same apparent phosphate contents were found either with or without the preliminary permanganate treatment.

TABLE 2  
*Preliminary experiments*

MATERIAL	ACID ADDED		K <sub>4</sub> Fe- (CN) <sub>6</sub>	MnSO <sub>4</sub>	pH	P <sub>2</sub> O <sub>5</sub> FOUND
	Before precipitation of iron	After precipitation of iron				
	cc.	cc.	cc.	cc.		mgm.
15 cc. L 1*	3.0	3.0	10.0	6.7	1.16	1.33
15 cc. L 1	3.0	3.0	10.0	4.8	1.16	1.33
15 cc. L 1	3.0	3.0	10.0	4.0	1.17	1.32
15 cc. L 4*	3.0	3.0	10.0	6.1	1.13	1.13
15 cc. L 4	3.0	0	10.0	6.1	1.44	1.11
15 cc. L 4	6.0	0	10.0	6.1	1.17	1.12
15 cc. L 4	3.0	3.0	10.0	9.9	1.17	1.13
15 cc. L 4	3.0	0	10.0	9.9	1.45	1.13
15 cc. L 4	6.0	0	10.0	9.9	1.18	1.11
Average for L 4						1.12
15 cc. L 4 + 0.10 mgm. P <sub>2</sub> O <sub>5</sub>	5.0	0	10.0	7.0	....	1.23
15 cc. L 4 + 0.25 mgm. P <sub>2</sub> O <sub>5</sub>	5.0	0	10.0	7.0	....	1.39
15 cc. L 4 + 0.50 mgm. P <sub>2</sub> O <sub>5</sub>	5.0	0	10.0	7.0	....	1.63

\* No preliminary permanganate treatment.

Table 2 shows the results of some of the preliminary experiments illustrating the foregoing points. It will be noted that in some cases the addition of the 2*N* sulfuric acid was made in two stages in order to ascertain whether any advantage resulted from carrying out the precipitation in a less acid solution than that finally produced.

#### *Method finally used*

Fifteen milliliters of soil extract, either with or without preliminary permanganate treatment, was diluted to about 40 cc. and neutralized with ammonia until a full orange color, but no precipitate, was developed. Five milliliters of 2*N* sulfuric acid was then added, followed by 10 cc. ferrocyanide

and 7 cc. manganese sulfate. After standing a few minutes the solution was diluted to 100 cc., filtered through a No. 30 Whatman paper, the first few milliliters being discarded, and aliquots of the filtrate were used for color development. The results obtained by this method are shown in table 3 and have been corrected for  $P_2O_5$  contained in the reagents.

### *Alternative methods*

Preliminary experiments indicated that the phosphoric acid isolated from soil extracts as ammonium phosphomolybdate could be determined colorimetrically. The following procedure, which includes Ward's method (6) of removing silica and organic matter, was finally adopted.

Soil extract, 20 or 25 cc., along with 2 cc. each of sulfuric, hydrochloric, and nitric acids, was evaporated on a water bath and then on a sand bath until distinct fumes of sulfur trioxide were evolved. Thereafter the residue was

TABLE 3

*Results, expressed as percentages  $P_2O_5$  in soil, obtained by the modified Warren and Pugh method (M. W. P.), the alternative method (A. M.), and the confirmatory methods (C. M.)*

METHOD	L1	L2	L3	L4	L5	L6	L7	L8
M. W. P.	0.109	0.099*	0.092*	0.093*	0.118*	0.108*	0.119*	.....
A. M.	.....	0.099	0.094	0.092	0.121	0.107	0.119	0.109
C. M.	.....	.....	.....	.....	0.115	0.104	.....	.....
	C1	C2	C3	C4	C5	C6	C7	C8
M. W. P.	0.098*	0.096	0.107	0.097	0.096	0.089	0.127	0.087
A. M.	.....	0.101	0.107	0.098	0.097	0.091	.....	.....
C. M.	.....	.....	.....	.....	.....	0.089	.....	.....

\* No preliminary permanganate treatment.

dissolved in water, and silica was removed by filtration. Phosphoric acid was precipitated from the filtrate in the manner described by Woy (8) for his first precipitation except that, to avoid any chance of incomplete deposition of the phosphomolybdate, the solution was heated for 2 or 3 hours and then allowed to stand overnight at room temperature. Next day, the precipitate was washed by decantation with the solution recommended by Woy, the decanted liquid being poured through a No. 30 Whatman filter paper. The precipitate was then dissolved by pouring 10 cc. of 1 in 10 ammonia, in three portions, through the filter into the beaker containing the bulk of the precipitate, and the filter was washed twice with water, once with very dilute acid, and, finally, twice with water. The ammoniacal solution and the washings were diluted to about 50 cc., acidified by the addition of 1 cc. of concentrated hydrochloric acid, and made up to 100 cc.

The solution so obtained was virtually iron-free and, as no precipitate formed even on standing several days, portions could be taken for color development,



by the usual method, when required. The results obtained in this way are shown in table 3 for comparison with the figures obtained by the modified Warren and Pugh method.

### *Confirmatory methods*

For additional confirmation, silica and organic matter were removed from three of the extracts (C6, L5, and L6) by double evaporation to dryness, in the presence of nitric acid, and baking. As titanium was known to be present, the silica residues were fused with sodium carbonate, the melt dissolved in hot water, iron and titanium removed by filtration, and silica again removed by double evaporation to dryness and baking. Phosphoric acid was estimated as follows: (a) In the case of C6, the two silica-free solutions were combined, and the total phosphoric acid was determined gravimetrically after double precipitation as ammonium phosphomolybdate, as described by Woy. (b) In the case of both the other extracts, phosphoric acid was determined separately, in each of the silica-free solutions, by colorimetric methods. After correction had been made for the phosphoric acid in the sodium carbonate, 12.5 per cent of the total phosphoric acid in L5 was recovered from the original silica residue, and the corresponding figure for L6 was 6.8 per cent.

The percentages of phosphoric acid found by these methods are included in table 3.

### EXAMINATION OF METHODS

In the preceding part of this paper, a modification of the Warren and Pugh method was described which, while retaining the simplicity and speed of the original method, appears to be applicable to a wider range of soils. This modified method was developed somewhat empirically. In the following pages, controlled experiments are described, and an attempt is made to ascertain the limitations of the method.

For the experiments, test solutions were prepared consisting of 4 cc. concentrated hydrochloric acid, 1.0–1.2 mgm.  $P_2O_5$ , and variable quantities of iron. These solutions were designed to correspond to 15 cc. of extract from a soil containing 0.083–0.100 per cent acid-soluble  $P_2O_5$  and, in some cases, known amounts of other elements were added before the solutions were submitted to the standard procedure, for isolating phosphate, already described. In all cases the procedure was the same, but the volumes of ferrocyanide, manganese sulfate, and acid were varied in different experiments.

### *Iron alone*

No special difficulty was encountered with iron alone, but it was noted that the amount of ferrocyanide required to precipitate iron indicated a precipitate with the composition  $FeHFe(CN)_6$  rather than  $Fe_4[Fe(CN)_6]_3$ . The latter composition would indicate that, to precipitate 0.1 gm. of Fe, 5 cc. of 10 per cent potassium ferrocyanide would be required, whereas it was found that even 6 cc. were not sufficient. This is interesting in view of the failure of the original

method (employing 6 cc.) to remove iron completely from the Nyasaland soil extracts, some of which contained as much as 0.097 gm. of iron in 15 cc.

When an excess of ferrocyanide had been used, the method yielded solutions which gave virtually no color when tested with thiocyanate. The importance of this complete removal of iron is illustrated by the following results of experiments in which color development was carried out in the routine manner with 0.04 mgm.  $P_2O_5$  standards but with variable amounts of iron:

Fe.....mgm.	0.000	0.010	0.013	0.020	0.025	0.040	0.050
Color.....per cent	100.0	99.6	98.9	99.2	97.5	97.2	92.5

### *Titanium*

Titanium forms an insoluble ferrocyanide and theoretically should be precipitated along with iron, but in practice it was found to be a limiting factor for the method.

Experiments with test solutions containing titanium and iron are summarized in table 4, and the following conclusions were reached:

The removal of titanium is rarely complete and becomes increasingly difficult as the Ti/Fe ratio, and to a lesser extent the absolute amount of titanium, increases.

The recovery of phosphoric acid is similarly controlled, and if the solution after filtration gives more than the slightest yellow color on addition of hydrogen peroxide, low results are to be expected.

The recovery of phosphoric acid is also controlled by the acidity. In general, the greater the amount of titanium, the greater is the acidity required to insure a good recovery.

With titanium present in appreciable quantity, it is important to use a considerable excess of ferrocyanide.

The method becomes unsatisfactory when much titanium is present. Thus in the presence of 0.1 gm. of iron, the practical limit, even with increased acidity and ferrocyanide, is probably about 17 mgm. titanium.

Analysis of two of the extracts of the Nyasaland soils indicated titanium contents of the order of 3.4 mgm. per 15 cc., and the iron present was of the order 0.06 to 0.10 gm. The result of experiment 4 (table 4), therefore, indicates that satisfactory results would be obtained with the extracts under similar conditions. It may also be noted that the higher acidity found necessary with this method is probably due to the presence of titanium in the extracts.

### *Vanadium*

An experiment in which vanadium (0.01 gm. vanadium chloride) was present along with iron and titanium gave, with the usual procedure, satisfactory recoveries of phosphoric acid. It was concluded, therefore, that vanadium did not upset the method.

### *Aluminum*

The presence of aluminum was found to have no effect on the modified method, but when test solutions with aluminum were treated by the original Warren and Pugh procedure, it was found that the pH of the solution produced

was greatly influenced by the quantity of aluminum present. A probable explanation is that, during the neutralization to pH 6.8, aluminum hydroxide is precipitated in the original method and, consequently, on subsequent acidification a portion of the acid is used up in redissolving the precipitate. With the modified method, neutralization is stopped before aluminum is precipitated,

TABLE 4

*Recoveries of 1.0 mgm.  $P_2O_5$ , from test solutions containing variable amounts of titanium and iron, after correction for the average blank of the reagents, i.e., 0.02 mgm.  $P_2O_5$*

EXPERIMENT NUMBER	TEST SOLUTION		REAGENTS USED			COMPOSITION OF SOLUTION PRODUCED					
	Ti	Fe	Acid	$K_4Fe(CN)_6$	$MnSO_4$	Fe*	Ti*	$K_4Fe(CN)_6$ *	pH	Normality	$P_2O_5$ found
	mgm.	mgm.	cc.	cc.	cc.						mgm.
1	3.4	51	8.0	6.0	3.0	—	—	—	1.11	.166	0.98
2			8.0	10.0	7.0	—	—	—	1.18	.164	1.00
3		101	6.0	6.0	5.0	++	nd	—	1.23	nd	0.82
4			6.0	10.0	7.0	—	nd	—	1.31	nd	0.98
5	6.9	53	5.0	10.0	7.0	—	+	—	1.34	nd	0.84
6			10.0	7.0	4.0	—	t	—	1.07	.204	1.00
7		103	6.0	6.0	5.0	++	nd	—	1.27	nd	0.80
8			6.0	8.0	4.9	—	+	—	1.36	nd	0.96
9			6.0	10.0	7.0	—	—	—	1.40	nd	0.96
10			6.0	12.0	9.0	—	t	—	1.40	nd	0.96
11			8.0	8.0	4.9	+	++	—	1.26	nd	0.91
12			8.0	10.0	7.0	—	t	—	1.28	nd	1.00
13			8.0	9.1	8.0	—	t	—	1.31	nd	1.01
14			8.0	10.0	5.0	—	t	—	1.25	nd	1.01
15			8.0	10.0	9.0	—	t	—	1.30	nd	1.00
16			10.0	10.0	7.0	—	t	—	1.20	.215	0.99
17	17.2	57	10.0	11.0	7.0	—	t	—	1.16	nd	0.58
18			12.0	12.5	9.5	—	+	—	1.06	.248	0.74
19		107	8.0	11.0	7.0	—	+	—	1.30	nd	0.90
20			10.0	15.0	9.0	—	+	—	1.21	.215	0.93
21			10.0	14.0	9.0	—	t	—	1.16	nd	0.97
22			12.0	15.0	9.0	—	t	—	1.04	.255	0.96

\* Colorimetric Tests: KCNS for Fe,  $H_2O_2$  for Ti, and iron for  $K_4Fe(CN)_6$ . + = definite color; t = trace of color; — = no color; nd = not determined.

and accordingly all the acid is available for increasing acidity, with the result that a definite volume of standard acid produces approximately the same pH independently of the aluminum present.

#### *Silica and organic matter*

Silica and organic matter were not found to cause any trouble in the analysis of the Nyasaland soils, but either or both may be of importance in other soil

types. The following suggestions for their removal are therefore made, the first two being available for citric acid extracts (6, 7):

Organic matter may be removed by permanganate as in the original Warren and Pugh method.

Organic matter and most of the silica may be removed in one operation by Ward's method (6), which has been described in connection with the precipitation-colorimetric method.

Removal of silica by evaporation and baking, although inadmissible when iron and titanium are both present, may be applied to solutions *after* the removal of these elements. This procedure was tried with some of the soil extracts, an aliquot, which would normally have been used directly for color development, being freed from silica and organic matter by double evaporation to dryness in the presence of nitric and hydrochloric acids, and the final residue, so obtained, dissolved in a little acid, and the resultant solution used for color development. It was found that only about 1 per cent of the  $P_2O_5$  was lost in the process.

### Acidity

The effects of variable quantities of 0.1*N* sulfuric acid on color development with 0.04 mgm.  $P_2O_5$  were found to be as follows:

0.1 <i>N</i> acid.....cc.	0	10	20	25	30	35	50
Color.....per cent	100.0	98.8	96.8	96.0	95.6	94.0	89.6

It therefore appears that, if underestimation of phosphate is to be avoided, the quantity of acid solution taken for color development must not exceed equivalence to 10 cc. of 0.1*N* acid. Table 4 indicates that the normality of the iron-free solution can be represented approximately by 0.021*s* (*s* = volume of 2*N* sulfuric acid), and the volume equivalent to 10 cc. 0.1*N* acid is the reciprocal of this normality. On the other hand, if 0.04 mgm.  $P_2O_5$  standards are used, the volume required for color development must be approximately 1/3*P*, where *P* = per cent HCl-soluble  $P_2O_5$  in the soil. Combining these results, appreciable underestimation of  $P_2O_5$  may be expected if  $P < 0.007s$ . Thus, when 8 cc. of acid are employed, appreciably low results may be expected, because of acidity, if the soluble  $P_2O_5$  in the soil is much under 0.056 per cent.

Experiments with test solutions containing iron and  $P_2O_5$  equivalent to 15 cc. of extracts from soils containing 0.021 and 0.0125 per cent  $P_2O_5$  resulted in recoveries equivalent to 0.020 and 0.120 per cent respectively. Greater accuracy was attained very simply, however, by adding equivalent amounts of acid to the 0.04 mgm. phosphate standard prior to color development. When this was done, virtually theoretical results were obtained. The alternative method of neutralizing the solutions yielded high recoveries, presumably because of the effect of neutral sulfates on color development reported by Chapman (2).

The foregoing calculations apply to extracts prepared by the particular method used for the Nyasaland soils, but analogous considerations can be applied to any type of extract.

### NOTES

1. *Filtration of ferrocyanide precipitates.* Ward (6) has reported difficulty in filtering ferrocyanide precipitates, but this has not been experienced when the following precautions

were taken: (a) a reasonable excess of manganese sulfate was used; (b) the filter paper (Whatman No. 30) was folded in the ordinary manner and the inner fold bent round spirally so as to avoid contact with the rim; (c) the filter was never filled above the level of the first filling; (d) the first few milliliters of filtrate (which usually had a blue color) were discarded.

2. *Small amounts of iron in extracts.* When iron is present in only small quantity, 0.05 gm. iron should be added as an indicator.

3. *Estimation of titanium.* The writer found that a convenient way of estimating titanium in extracts was to neutralize to the orange iron color with ammonia and boil with excess of sodium thiosulfate for 5 minutes. The precipitate of aluminum and titanium hydroxides was well washed with hot water and redissolved in hot 1:3 sulfuric acid. The solution was allowed to stand overnight to flocculate suspended sulfur, and after filtration and dilution to a definite volume, aliquots were used for colorimetric titanium estimation by hydrogen peroxide. Recoveries of about 99 per cent were obtained.

4. *Large amounts of titanium.* When titanium is present in excess of about 17 mgm., the method becomes unreliable, but in such cases, the alternative precipitation-colorimetric method is still applicable. When a test solution containing 126 mgm. iron, 69 mgm. titanium, and 1.0 mgm.  $P_2O_5$  was employed in the latter method, a recovery of 1.06 mgm.  $P_2O_5$  was obtained. This high recovery was thought to be due to traces of phosphate in the titanium chloride used.

#### SUMMARY

A modification of the Warren and Pugh method for determining phosphoric acid is described which, without sacrifice of speed or simplicity, gave satisfactory results with extracts of certain Nyasaland soils when the original method had failed. Further investigation of the scope of the method indicates that, with some simple modifications, it can be applied to extracts with a very considerable range of compositions, including those obtained in determinations of available phosphates, but that low estimates of  $P_2O_5$  may be expected when appreciable quantities of titanium are present.

An alternative method is also described which, though considerably more tedious, gives almost identical results when both methods are applicable and appears to be unaffected by relatively large quantities of titanium.

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# THE OCCURRENCE OF SELENIUM IN UTAH FORAGE PLANTS

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Selenium is present in the soil in varying quantities; in certain localities, the vegetation contains sufficient to be toxic when eaten by animals. Several states, as well as the United States Department of Agriculture, have realized the economic importance of the selenium problem and have made studies regarding the occurrence and distribution of this mineral in soils and plants. Our knowledge of the selenium content of Utah soils and of Utah-grown plants is meager. Hence, this study was undertaken to determine the selenium content of typical forage plants collected from various parts of the state to learn whether they carry sufficient selenium to injure the animals which feed upon them.

## OCCURRENCE OF SELENIUM

Three methods have been used to determine the geographical distribution of selenium: (a) the analysis of soils for selenium, (b) a study of geological formations from which the soils developed, and (c) a study of native vegetation found growing on seleniferous soils. Beath,<sup>1</sup> Eppson, and Gilbert (2) reported that selenium occurs in varying quantities through Cretaceous and Tertiary shales. Later they (6) found additional geological formations which are capable of supporting seleniferous vegetation. Of great importance in connection with locating seleniferous areas has been the discovery by Beath and co-workers (1, 3, 4) that certain native selenium-bearing plants may be used as indicators. As these native plants can be relied upon to contain significant amounts of selenium during all or a major part of their annual growth, their occurrence indicates the presence of selenium in soils upon which they grow. Beath, Gilbert, and Eppson (5) list the primary group of selenium indicator plants as certain species of the following genera: *Stanleya*, *Oenopsis*, *Xylorrhiza*, and *Astragalus*.

The existence of seleniferous areas has been established in parts of 16 western states, including Wyoming, Arizona, South Dakota, Utah, Colorado, California, Idaho, Montana, Nevada, New Mexico, Oregon, Texas, Kansas, North Dakota, Nebraska, and Oklahoma.

Byers (7) was the first to analyze plant samples collected within the state

<sup>1</sup> The authors wish to acknowledge suggestions and help received on methods from O. A. Beath, of the Wyoming Agricultural Experiment Station.

of Utah. Later Byers, et al. (8) reported the results of a survey of parts of Utah which established the existence of seleniferous areas. Beath, Gilbert, and Eppson (5) found that a particular shale formation in Provo Canyon, Wasatch Mountains, Utah, is among the most highly seleniferous geological formations in the western United States.

They (6) also found from the analysis of a number of seleniferous indicator plants that selenium occurs in the following areas within the state of Utah: in the eastern part near Cisco, in scattered patches from Cisco to Price and from Cisco to Moab, in Box Elder County in northwestern Utah, in Provo Canyon, and in Washington County.

#### METHOD OF INVESTIGATION

The modifications (9, 19) of the Robinson, Dudley, Williams, and Byers (RDWB) (20) methods and modifications (13, 14) of the Horn (10) were checked. As a result of this preliminary work the method selected for the determination was as follows: a 5-gm. plant sample was digested until colorless in a Kjeldahl flask containing 50 cc. of concentrated sulfuric acid and 0.2 to 0.4 gm. of mercuric oxide. The Kjeldahl flasks were cooled, and the material was transferred quantitatively to a 50-cc. volumetric flask and diluted to volume with concentrated sulfuric acid. A 10-cc. portion was placed in a dry test tube and centrifuged until the solution was clear. Three drops of saturated codeine hydrochloride were added to each tube and allowed to stand in a dark cupboard overnight. A blue color developed in the tubes containing selenium. A rough quantitative determination was made after 24 hours by comparing the tubes with standards which had been similarly prepared.

The smallest quantity of selenium which could be detected by this method was 0.002 mgm., equivalent to 0.4 p.p.m. When no color was detected the result was recorded as negative, and quantities between 0.4 and about 2.5 p.p.m. were recorded as traces. The upper limit of a trace was chosen as 2.5 p.p.m. because quantities less than this have a considerable percentage of error even when determined by the modified RDWB method. Plants carrying less than 4 p.p.m. of selenium are not considered toxic to animals which feed upon them (7).

Samples which were found to contain selenium in quantities greater than about 2.5 p.p.m. were checked by the following method: A 10-gm. sample was digested with 10 gm. of a catalytic mixture (100 gm. of anhydrous sodium sulfate, 3 gm. of mercuric oxide, and 2.2 gm. of anhydrous copper sulfate) and 90 to 100 cc. of concentrated sulfuric acid in a special Pyrex distillation apparatus. A small flask, such as a 150-cc. fat-extraction flask, containing a small amount of water was used as a receiver, the tip of the condensor being immersed in the water.

The distillation flask was slowly heated until danger of foaming was past and then strongly heated. The material in the flask was digested until the sulfuric acid was clear. On completion of the digestion the flask was allowed

to cool, and the material in the receiver was added through the thistle tube to the digested material in the distillation flask. The flask was again cooled and 12 to 20 cc. of a 1 to 10 bromine-hydrobromic acid added. The mixture was distilled into a flask containing a little water until virtually all of the hydrobromic acid was distilled over, indicated by a lightening of the color of the material in the Kjeldahl flask. The distillate was filtered through a Gooch crucible to remove waxy material and was decolorized with sulfur dioxide, which was allowed to run into the solution for a minute or two after decolorization. About 0.2 to 0.4 gm. of hydroxylamine hydrochloride was added and the mixture heated on a steam bath for 1 hour. After standing overnight the precipitated selenium was filtered through a small Gooch crucible and washed with water to remove any hydrobromic acid.

The selenium was dissolved in about 15 cc. of hot concentrated nitric acid diluted with water and filtered from the asbestos by suction. The nitric acid solution was evaporated on a steam bath until nearly dry. The residue was dissolved in dilute 1 to 10 nitric acid to which about 0.2 to 0.3 gm. of urea had been added to destroy the lower oxides of nitrogen. A few small crystals of potassium iodide were added to this solution, and the liberated iodine was titrated with a standard thiosulfate solution using starch indicator. A satisfactory thiosulfate solution was one of such strength that 1 cc. is equivalent to about 0.1 mgm. of selenium. The results obtained by this method were recorded as parts per million.

Typical forage plants were analyzed. They were collected from grazing districts throughout the state. Each sample was a composite collected from many plants within the specific area. Mature plants were collected, and only that part consumed by animals was used. They were carefully composited, air dried, and finely ground for analysis (11).

The districts investigated were as follows: 1. Trout Creek area in western Utah. This area forms the western half of Juab County, southwestern Tooele County, and northwestern Millard County. 2. Pine Valley and Antelope Valley area in southwestern Utah. This includes Beaver County and the southern half of Millard County. 3. A district in Cache County, northeastern Utah, which includes a part of Cache National Forest Reserve and points near Logan.

#### RESULTS

A list of plant species from the Trout Creek area, the percentage of plants carrying selenium, and the relative quantities carried are given in table 1.

Eighty-two per cent of the 40 plants analyzed from the Trout Creek area were found to contain selenium. This large percentage indicates that selenium is very generally present in the soil of this area. Only 20 per cent of the plants, however, contained more than 2.5 p.p.m. of selenium.

Byers (7) assumes 4 p.p.m. of selenium as a tolerance limit and considers 5 p.p.m. as potentially dangerous. Moxon (19) asserts that chronic selenium



poisoning results when an animal consumes feed containing 5 to 40 p.p.m. of selenium for several days or weeks.

Plants from the Trout Creek area that were found to contain over 2.5 p.p.m. of selenium are listed in table 2. Even if the animal ration consisted entirely

TABLE 1

Number of samples, percentage of samples containing more than 2.5 p.p.m. of selenium, percentage containing a trace of selenium, and percentage containing no selenium in 10 plant species from the Trout Creek area

NAME		NUMBER OF SAMPLES	PERCENTAGE OF SAMPLES WITH		
Scientific	Common		More than 2.5 p.p.m. selenium	Trace of selenium	No selenium
<i>Atriplex canescens</i>	Fourwing saltbush	3	0	100	0
<i>Atriplex nuttallii</i>	Salt sage	3	0	100	0
<i>Artemisia tridentata</i>	Blue sage	6	17	83	0
<i>Gutierrezia sarothrae</i>	Matchweed	6	0	83	17
<i>Ephedra nevadensis</i>	Brigham tea	5	20	60	20
<i>Eurotia lanata</i>	White sage	2	0	50	50
<i>Atriplex confertifolia</i>	Shadscale	5	40	40	20
<i>Juniperus utahensis</i>	White cedar	3	67	33	0
<i>Salsola pestifer</i>	Russian thistle	3	33	33	33
<i>Chrysothamnus viscidiflorus</i>	Yellowbrush	4	25	25	50
Average.....			20	62	18

TABLE 2

Plant samples from Trout Creek area containing more than 2.5 p.p.m. of selenium

SCIENTIFIC NAME	SELENIUM
	p.p.m.
<i>Atriplex confertifolia</i> .....	4.8
<i>Salsola pestifer</i> .....	4.4
<i>Ephedra nevadensis</i> .....	4.4
<i>Artemisia tridentata</i> .....	3.7
<i>Juniperus utahensis</i> .....	3.7
<i>Juniperus utahensis</i> .....	3.6
<i>Atriplex confertifolia</i> .....	2.6
<i>Chrysothamnus viscidiflorus</i> .....	2.6

of the plants given in table 2, it would still be under the tolerance limit, and poisoning would probably not result.

A list of plant species from the area about Pine Valley and Antelope Valley, the percentage of plants containing selenium, and the relative quantities carried are given in table 3.

Seventy-one per cent of the plants from this area were found to contain selenium as compared to 82 per cent from the Trout Creek area. Eight per cent of plants from this area as compared to 20 per cent from the Trout Creek area contained quantities of selenium greater than 2.5 p.p.m.

The plants from this area containing more than 2.5 p.p.m. selenium were as follows: a sample of *Atriplex confertifolia*, 5.4 p.p.m., and a sample of *Ephedra nevadensis*, 2.6 p.p.m. As these plants would constitute only a part of the animal's ration, their selenium content would not be sufficient to cause poisoning.

Plants from the Cache National Forest Reserve and places near Logan were found to contain little or no selenium.

The following plant samples contained traces of selenium: two *Vicia americana*, one *Astragalus utahensis*, and one *Artemisia frigida*. These plant species

TABLE 3

Number of samples, percentage of samples containing more than 2.5 p.p.m. of selenium, percentage containing a trace of selenium, and percentage containing no selenium in a few plant species from the Pine Valley and Antelope Valley area

NAME		NUMBER OF SAMPLES	PERCENTAGE OF SAMPLES WITH		
Scientific	Common		More than 2.5 p.p.m. selenium	Trace of selenium	No selenium
<i>Artemisia nova</i>	Curley sage	4	0	75	25
<i>Ephedra nevadensis</i>	Brigham tea	7	14	72	14
<i>Atriplex confertifolia</i>	Shadscale	5	20	60	20
Other species*		8	0	50	50
Average.....			8	63	29

\* One or two samples of each of the following species: *Atriplex canescens* (fourwing saltbush), *Atriplex nuttallii* (salt sage), *Artemisia tridentata* (blue sage), *Juniperus utahensis* (white cedar), *Chrysothamnus viscidiflorus* (yellowbrush), and *Eurotia lanata* (white sage).

are found to contain large quantities of selenium when grown on seleniferous soils. This indicates that selenium is present in small quantities and would not be absorbed by most forage plants.

The following plant samples from this district were found to contain no selenium: *Chrysothamnus viscidiflorus* (yellowbrush), *Chrysothamnus nauseosus* (rabbitbrush), one of *Atriplex nuttallii* (salt sage), one of *Prunus melanocarpa* (chokecherry), one of *Prushia tridentata* (bitterbrush), one of *Zygadenus gramineus* (death camas), one of *Gutierrezia sarothrae* (matchweed), one of *Thermopsis montana*, one of *Agropyron pauciflorus* (slender wheatgrass), and one of *Agastache urticifolia* (horsemint).

Three samples of *Artemisia nova* from the grazing area of Skull Valley in eastern Tooele County were found to contain no selenium.

The main plant species from all the districts investigated, the percentage

of plants carrying selenium, and the relative quantities carried are given in table 4. The 13 species analyzed were found capable of absorbing selenium, but they varied in amounts absorbed.

TABLE 4

Number of samples, percentage of samples containing more than 2.5 p.p.m. of selenium, percentage containing a trace of selenium, and percentage containing no selenium in plant species from all the districts investigated

NAME		NUMBER OF SAMPLES	PERCENTAGE OF SAMPLES WITH		
Scientific	Common		More than 2.5 p.p.m. selenium	Trace of selenium	No selenium
<i>Atriplex canescens</i>	Fourwing saltbush	4	0	100	0
<i>Gutierrezia sarothrae</i>	Matchweed	6	0	83	17
<i>Artemisia nova</i>	Curley sage	4	0	75	25
<i>Artemisia tridentata</i>	Blue sage	7	14	72	14
<i>Ephedra nevadensis</i>	Brigham tea	11	18	64	18
<i>Atriplex nuttallii</i>	Salt sage	5	0	60	40
<i>Juniperus utahensis</i>	White cedar	5	40	60	0
<i>Artemisia spinescens</i>	Bud sage	4	0	50	50
<i>Eurotia lanata</i>	White sage	4	0	50	50
<i>Chrysothamnus nauseosus</i>	Rabbitbrush	4	0	50	50
<i>Atriplex confertifolia</i>	Shadscale	11	36	46	18
<i>Salsola pestifer</i>	Russian thistle	3	33	33	33
<i>Chrysothamnus viscidiflorus</i>	Yellowbrush	7	14	14	72

TABLE 5

Amounts of selenium and percentages of sulfur in 8 samples of shadscale

SAMPLE OF SHADSCALE	SULFUR	SELENIUM
	<i>per cent</i>	<i>p.p.m.</i>
1	0.247	trace
2	0.267	4.8
3	0.272	0
4	0.290	trace
5	0.290	5.4
6	0.309	trace
7	0.420	0
8	0.560	2.6

Beath, Gilbert, and Eppson (5) list *Atriplex canescens* and *Atriplex nuttallii* as representative species that may be seleniferous and call them "convertor" plants. They found fourwing saltbush to be somewhat more dependable than salt sage as a selenium-absorbing plant under the same soil conditions. They have not found *Atriplex confertifolia*, normally, to be seleniferous.

No distinction could be made between the so called "convertor" plants and some of the other plant species. *Atriplex confertifolia* and *Juniperus utahensis*, which are not listed as "convertor" plants, were found to be slightly more seleniferous than any other plant species analyzed.

There is a controversy among investigators (12, 16, 17, 18, 19) concerning the use of sulfur to prevent absorption of selenium; hence, it is interesting to compare the sulfur and selenium content of these plants (11). In table 5 are given the selenium and the sulfur contents of several samples of shad-scale.

Results similar to those given for shadscale in table 5 were found for other plant species. There is no apparent relationship between the sulfur content and selenium content of forage plants found growing on different soils (15).

#### CONCLUSIONS

The results obtained in this work indicate that small amounts of selenium are present in the majority of forage plants in the Trout Creek area and in the Pine Valley and Antelope Valley area, whereas the forage plants from the Cache National Forest Reserve area are almost free from selenium.

The forage plants from the areas investigated do not contain sufficient selenium to be considered toxic to animals which feed upon them.

There is no apparent relationship between the sulfur and selenium content of forage plants from the areas studied.

#### SUMMARY

This study was undertaken to determine the occurrence of selenium in typical forage plants collected from various parts of Utah and to learn whether these plants carry sufficient selenium to be toxic to animals.

Plants from the different areas were tested for their selenium content by a colorimetric method using codeine, and those plants which contained more than about 2.5 p.p.m. were further tested by the volumetric modified method of Robinson, Dudley, Williams, and Byers.

Eighty-two per cent of the forage plants analyzed from the Trout Creek area and 71 per cent of the plants from the Pine Valley and Antelope Valley area were found to contain small amounts of selenium. Twenty per cent of the plants from the Trout Creek area contained over 2.5 p.p.m. as compared to 8 per cent of the plants from the Pine Valley and Antelope Valley area. The majority of plants from the Cache National Forest Reserve did not contain selenium, and the vetches, which are usually highly seleniferous, contained only a trace. All of the plant species analyzed were found capable of absorbing selenium and were found to vary in the amounts absorbed. The most selenium found in any plant was 5.4 p.p.m. There was no apparent relationship between the amount of selenium and the per cent of sulfur in the plants.

It is concluded that small amounts of selenium occur in forage plants in some areas of the state of Utah, but none of the plants studied contained sufficient selenium to be considered toxic.

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# THE AVAILAMETER AND ITS USE IN SOIL MOISTURE CONTROL: I. THE INSTRUMENT AND ITS USE<sup>1</sup>

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Irrigation studies conducted at Medford, Oregon, since 1930 have shown the advisability of closely regulating soil moisture in the major portion of the rooting zone of pear trees. When the average soil moisture in the major rooting zone of pear trees on heavy clay soils, the upper 3 feet (1), was maintained high in the available range, larger fruit and greater yields resulted than when the soil moisture was allowed to decrease much below the approximate midpoint of the available range (9, 10). When this occurred, rate of fruit, shoot, limb, and trunk growth, and duration of stomatal opening decreased. Soil moisture investigations have also shown a marked lack of penetration of irrigation water into the lower part of the rooting zone in many soils of this area.<sup>3</sup> Soil moisture in the major rooting zone should be replenished to field capacity at each irrigation; therefore, knowledge of soil moisture conditions immediately following each irrigation is needed.

In practical irrigation it is desirable to know the available water holding capacity of the soil, represented by the difference between the upper limit (field capacity) and the lower limit (wilting percentage) of usable soil moisture. We express available soil moisture as a percentage of this capacity, zero availability being at the wilting percentage and 100 per cent availability being at field capacity.

<sup>1</sup> This paper reports investigations formerly conducted under a cooperative agreement between the Bureau of Agricultural Engineering and Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station, and more recently conducted under a cooperative agreement between the U. S. Bureau of Plant Industry and the Oregon Agricultural Experiment Station. Presented for publication as Technical Paper No. 339, with the approval of the director, as a contribution of the Medford Branch, Oregon Agricultural Experiment Station.

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## CRITERIA OF IRRIGATION NEED

As clear-cut results of the Medford studies accumulated, it became evident that means were needed of applying them to commercial orchards. Numerous methods have been advanced for determining in commercial practice when irrigation should be applied to orchards. Taylor and Furr (21) have suggested that for citrus on some soils irrigation dates may be best determined by measurements of fruit growth rate, irrigation being applied when the rate begins to slacken. They cite other soils in which soil moisture samples may prove the better guide. Magness, Degman, and Furr (14) suggested the use of fruit growth measurements in determining the need for irrigation in eastern apple orchards. Veihmeyer and Hendrickson (22) have recommended that orchards be irrigated at about the wilting percentage or whenever broad-leaved plants in orchards, by wilting, give evidence of suffering from lack of soil moisture. They caution growers against delaying irrigation until the plants actually wilt, particularly if the orchard be large and the irrigation stream small. Their recommendation has not been applied locally to pear orchards on clay soils because the trees suffer very seriously for lack of water, as shown by fruit growth measurements, for days or even weeks before weeds or trees show any readily visible sign of wilting. This loss in fruit volume is not regained after irrigation, as has been shown in the case of citrus (21). Veihmeyer and Hendrickson believe that soil samples, when regularly taken, give growers reasonably accurate advance knowledge of probable irrigation dates.

It has also been suggested that pear orchards, if irrigated at regular intervals, say approximately every 30 days, will produce maximum crops, and the expense of soil moisture sampling might be avoided. Work (24) showed that this suggestion is impractical and said:

These results show clearly that the maintenance of soil moisture within the upper half of the available range [shown to be necessary for satisfactory pear production on the heavy soil of the Medford Branch Station] requires very different irrigation schedules on different orchards. The data indicate that because of wide differences in soil depth, soil type, tree size and vigor, and because of wide variations in evaporating power of the air from month to month and also from year to year, it is quite impracticable to adopt any uniform irrigation program either as to date of application of first or last irrigation, interval between irrigations, or number of irrigations.

... It appears to us, after several years work, that actual determinations of the moisture content of the soil itself must be carried on if the most efficient use of soil, water, and trees is to be made.

Actual determination of soil moisture in the major part of the root zone [the upper three feet with pears on heavy clay soil] affords at least four specific and necessary indications for the conduct of orchard irrigation. First, it indicates exactly how much soil moisture is available for use by the trees. Second, by periodic sampling at intervals of say a week to ten days, the orchardist knows how rapidly soil moisture is being lost and can anticipate very closely the time when soil moisture will be seriously depleted. Third, by taking samples after irrigation, the orchardist may learn to what depth and in what amount irrigation water penetrated and has a measure of the efficiency of his irrigation methods. Fourth, knowing his

soil moisture condition and the rate at which moisture is being lost, the orchardist can more intelligently coordinate other orchard operations, such as spraying, blight control, harvest, etc., with irrigation, and is in a much better position to judge whether irrigation, or some other equally important orchard operation should have the right of way.

#### SHORT-CUT SOIL MOISTURE DETERMINATION

To determine whether practical irrigation in commercial pear orchards could be based on detailed soil moisture sampling, an orchard soil moisture control project was initiated in 1936 at Medford (23, 24). In cooperation with other public agencies and with pear growers, the program was continued for 3 years. The total area comprised about 500 acres, on 10 different soil types, with a wide variation in topography, soil depth, tree size, and irrigation facilities. The growers found that with soil moisture data furnished at short intervals they usually could schedule their irrigations so as to maintain highly available soil moisture without serious interference with other orchard operations. In many cases inadequate penetration of irrigation water into the soil was very strikingly shown. The project early demonstrated need for a quicker, simpler, and less expensive method of soil moisture determination than that of oven drying. Accordingly, in 1937 a study was initiated to find a practical method of rapid soil moisture determination.

Any really satisfactory method of soil moisture determination for use in commercial irrigation practice should meet the following requirements:

It should give satisfactory results when used by an unskilled farm operator, and variation in the human element should have a minimum effect on the results.

Determinations should require a minimum of time and be made directly in the field.

The apparatus should be light, compact, and rugged, with a minimum of loose parts, and not sensitive to variations in slope of ground on which it might be placed.

Supplemental calculations required in determining availability of moisture to the crop should be eliminated or reduced to a minimum.

The method should allow variation in degree of refinement of results, depending on the need of the grower.

The apparatus should be of low cost to allow its use by the ordinary farmer.

Various short-cut methods of soil moisture determination have been studied. McCorkle (13) obtained promising results by measuring electrical resistance of soil between four similar electrodes permanently set in the soil. Variation in contact resistance was eliminated by setting up simultaneous equations, which included the resistance measured between the electrodes. A moisture meter described by Ehrenburg (6) employed direct current resistance measurements on soil samples carefully tamped into a cup containing electrodes at top and bottom. Emmert (7) measured the temperature rise of a 1-gm. soil sample to which 2 cc. of concentrated sulfuric acid had been added, the heat being developed from the action of the acid on the soil water. The higher the soil moisture content, the higher the temperature of the reaction that was noted. Good results with this method depend on accurate weighing and measurement of the very small samples. Bouyoucos (2, 3) extracted moisture from soil sam-



ples by treating them with alcohol and then burning the alcohol. Moisture from the soil was partly taken up by the alcohol and removed finally by evaporation. Weighing of the soil sample before and after drying is necessary to obtain the contained moisture. On heavier soils several drying cycles were found necessary to ensure complete loss of moisture. Bouyoucos and Mick (4) have reported promising results from resistance measurements on plaster of paris blocks permanently buried in the soil in such a manner as to reflect the moisture of the immediately surrounding soil. The details of this method were not available at the time the availameter investigation was carried out.

Heck (8) and Rogers (18) describe a soil moisture meter consisting of a water-filled porous clay pot buried in the soil and connected to a manometer or vacuum gauge. The soil surrounding the pot has an attraction for the water in the pot, the intensity of which depends on the soil moisture content and is measured by the vacuum gauge. As the moisture content of the soil later is increased by rain or irrigation, water is drawn from the soil into the pot and a corresponding decrease in vacuum is measured on the gauge. The principle of the tensiometer apparatus developed by Richards and Neal (16) for capillary potential and moisture flow measurements is similar. When correlated with the moisture content of the surrounding soil, this type of instrument apparently gives reasonable indication of moisture conditions in the immediately surrounding soil, as tests by Richards and Lamb (17), and by Staebner (20) show.

Shaw and Baver (19) have shown that heat conductivity of a given soil is largely influenced by contained moisture and that a method of measuring such conductivity apparently can be developed to give a reliable indication of soil moisture. Edlefsen (5), in a review of the literature on dielectric determination of moisture in various materials, discussed the application of dielectric measurements to soil moisture determination. J. E. Fletcher, of Soil Conservation Service, has made a preliminary report of encouraging progress on the dielectric measurement of plaster of paris condensers permanently buried in the soil for indication of soil moisture conditions.

Early in 1937 the writers experimented on measurements of electrical resistance of soil cores as obtained by the King tube, but results were unsatisfactory, apparently largely because of the difficulty in obtaining a constant contact condition between the electrodes and the soil. Polarization of the soil solution at the soil-electrode interface was thought to be a particularly troublesome factor.

None of these short-cut methods appeared to satisfy all the requirements for commercial soil moisture determination; consequently a different technique was developed which will be described in detail.

#### THE SOIL MOISTURE AVAILAMETER

It is generally observed that the higher the moisture content of soil, the more plastic and less stable it becomes when subjected to a deforming force. In

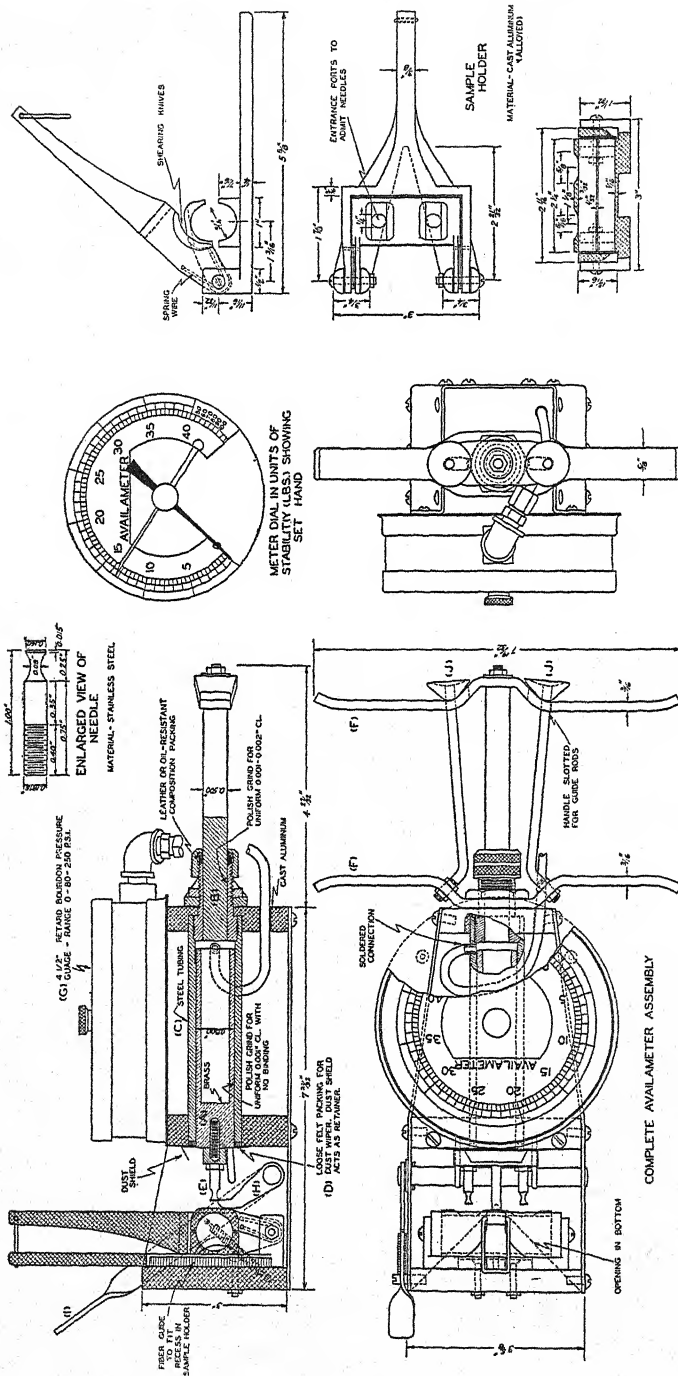
other words, the force necessary to cause a given deformation in soil of a given compaction increases as the moisture content decreases. This phenomenon is very marked in clay adobe soils. Stability, or resistance to deformation, of soil cores was determined by loading a plunger or needle footing of definite diameter until a predetermined penetration into the soil was obtained. Promising results were obtained when these stability measurements were correlated with the moisture contents of the sample cores. It was later found that Proctor (15) had made use of this soil moisture-plasticity relation in his compaction control work on rolled-earth fill construction. He showed that loose soil when compacted in a small cylinder or cup offered increased resistance to the penetration of a standardized plasticity needle as the soil moisture was decreased and suggested that such a method could be used for estimating soil moisture in earthwork construction once a moisture-plasticity curve had been prepared. L. H. Mitchell, of the U. S. Bureau of Reclamation, has made use of the Proctor soil needle in conjunction with a probe to determine the penetration of irrigation water.

Preliminary studies showed that strict control of the rate of load application was necessary to ensure satisfactory results, as variations in this rate resulted in corresponding variations in recorded readings. Several forms of apparatus were tried before a satisfactory device was found. Since determinations can be interpreted directly in terms of available soil moisture, we have chosen the name "Soil Moisture Availability Meter" to describe this instrument and have shortened this to "Availameter." The availameter is essentially an instrument for measuring the plasticity, stability, or degree of hardness of a soil sample. The present form of this device requires an undisturbed soil core of an approximately uniform  $\frac{3}{4}$ -inch diameter readily obtained with the King soil tube, using a slightly constricted cutting point.

#### *Description of the availameter*

The instrument proper, detailed in figure 1, consists of two opposing pistons, (*A*) and (*B*), with a connecting cylinder (*C*), to which is connected a pressure gauge (*G*). The pressure distributing medium between the two pistons is S.A.E. No. 10 lubricating oil. Piston *A* and cylinder *C* are very finely ground and fitted to a minimum clearance for free action so as to be sensitive to a very slight change of pressure. The loading piston (*B*) has an end area approximately 0.4 that of *A* and operates through a leather packing gland. A negligible leakage of oil occurs from the large nonpacked piston (*A*), sufficient for lubrication of the sliding surface and the loose felt collar packing (*D*) which prevents entrance of dust and grit. Recharging, if necessary, is accomplished by removing piston *B* and adding the oil through the cylinder.

The two plungers or needles (*E*) attached to the large piston each have a diameter of 0.160 inch and other dimensions as shown. This form has been tentatively adopted as standard. Stainless steel has proved most satisfactory, since brass or bronze wears badly and ordinary steel may rust.



The sample holder, also detailed in figure 1, cast from an aluminum alloy, grips the soil core, shears off the excess material at either end with a slight lateral compressive action and restrains it under the action of the needles. Two small portions of the soil core are exposed to the action of the needles through the two small openings or ports shown in the top leaf of the sample holder. The  $2\frac{1}{4}$ -inch length of core was adopted as being a satisfactory dimension for the various soil conditions found in this locality.

#### *Operation of the availameter*

The term "*soil stability*" or "*stability*" as used in this paper is defined as the *resistance in pounds offered by the soil to penetration of the standard needles to a depth of 0.20 inch* as measured by the availameter. If the moisture content-stability relationship is known for any particular soil, the stability determinations may be converted into, or readings may be made directly in, terms of moisture content or available moisture content as desired.

In operation, a soil sample is placed in the sample holder, clamped there, and the holder dropped into position in the instrument. Sufficient pressure is slowly applied by gripping the handles (*F*) with both hands to force the needles into the soil to a depth of 0.20 inch. The pressure gauge (*G*) indicates the fluid pressure behind piston *A* and since the gauge is calibrated directly to read pounds of force applied to the needles, the soil stability is read directly from the dial. The gauge is equipped with a rider pointer which records the maximum force applied and is reset to zero at the start of each determination.

As the shoulder is 0.25 inch from the point, the 0.20 inch depth of penetration of the needles into the soil can be judged with sufficient accuracy by noting the position of the shoulder with respect to the soil core. The proper rate of movement of the needles into the soil core is that which results in the desired penetration with the lowest loading pressure. A few practice trials will enable the operator to get the "feel" of the action so that a minimum reading is obtained at each determination. Two seconds is about the proper interval in which to effect needle penetration. By watching the action and position of the needles and catching the pointer on the gauge dial "out of the corner of the eye," one will find that the ultimate reading will be nearly reached before the penetration is appreciable and that the needles will then move into the soil with only very slight pressure increase. A quick thrust is improper, as it will cause a greater loading than is necessary. By developing the habit of watching the gauge dial as well as the advance of the needle footings, the operator can detect hidden gravel or sand particles by the increasing force necessary to advance the needles. Such a sample should be turned over in the holder and tried again. The holder cam (*H*) with connecting handle (*I*) keeps the sample holder in place (fig. 1) and is also used to extract the needles from hard dry samples. With moist samples, the return of piston *A* and extraction of needles is accomplished by placing thumbs on buttons *J* and returning handles *F* to starting position. With drier samples there is a possibility of air being drawn past piston *A* if the holder cam is not used.

## ORCHARD SOIL SAMPLING FOR AVAILAMETER MOISTURE DETERMINATION

With the soils so far tested in the Medford area, having moisture contents within the available range, very little difficulty has been experienced in obtaining soil cores in a satisfactory condition for use in the instrument. It should be emphasized that thus far determination of moisture-stability relationships has been largely confined to medium to heavy soil types which have given cohesive cores at all moisture contents. It seems likely, however, that moisture (stability) determination of light to very light soils may be made satisfactorily only within the moisture range where cohesive cores can be obtained with the soil tube. Some trouble may also be experienced with the present form of apparatus on soils containing more than small amounts of coarse sand and gravel.

In the soil moisture work on commercial orchards at Medford, at least five sampling locations were usually selected, uniformly distributed in each orchard block of 10 to 15 acres. Each location represented a different distance out from the tree within the root zone and a different area of the orchard. A greater number would have been desirable if additional sampling time had been available. Where soil lacks uniformity, sufficiently reliable average moisture content may not result from averaging samples from only five locations.

## SOIL STABILITY AS A DIRECT MEASURE OF SOIL MOISTURE AND MOISTURE AVAILABILITY

Work with the availameter was started with the aim of finding a satisfactory method of estimating the soil moisture content. As the study progressed, it was found that stability determinations could be readily interpreted directly in terms of the availability of moisture to crops. Though knowledge of actual moisture content is essential for a quantitative study of irrigation problems, data on availability alone are reasonably satisfactory for routine irrigation of crops.

Irrigation water normally should be applied whenever the available moisture in any part of the root zone has been depleted to the point where crop growth is impaired. To one planning soil moisture sampling as a guide to irrigation needs, determination of this soil moisture condition is of primary interest. In our studies, pear trees on clay soils have shown important reduction in responses, including size and yield of fruit, when the average available moisture content of the major part of the rooting zone has been reduced below about the midpoint of the available range. This soil moisture condition in these soils corresponds consistently to a soil stability of around 12 to 15 pounds.

Recent studies by Lewis (12) indicate that a given rate of capillary moisture movement toward the absorbing roots can be maintained only as long as a sufficiently high capillary moisture gradient is available. As moisture in the soil is extracted, the steepness of the gradient eventually may become definitely limited, and an average soil moisture condition is reached where

moisture is no longer available at a rate sufficient to maintain maximum crop growth. Such an average soil moisture content may be thought of as the point of stress, or "stress point," defined by Lewis (11). The point at which stress occurs varies widely, particularly in the heavy soils, with variation in weather. On hot, dry, windy days transpiration is greater than during cool weather, and consequently higher rates of capillary moisture movement are necessary. Practically, however, the stress point can be regarded as a more or less definite average soil moisture condition under usual or average weather conditions.

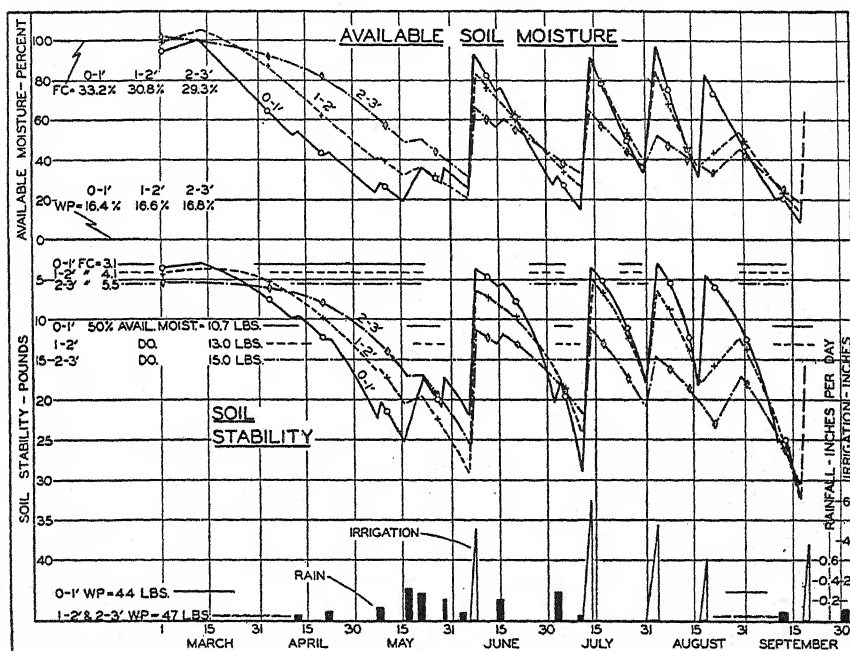


FIG. 2. AVAILABLE SOIL MOISTURE AND SOIL STABILITY FOR EACH OF THE UPPER 3 FEET IN A PEAR ORCHARD NEAR MEDFORD, OREGON, 1939

This stress point is held by workers in other localities to be lower in the available range than has been shown to be the case with the heavy Medford soils. Most growers recognize, however, that such a stress condition exists, and having once determined or estimated the corresponding stability for their particular soils, they will find that the availameter can serve as an excellent indicator of moisture needs of crops without calibration to actual moisture content. Furthermore, stability measurement offers a rapid and easily understood means of following rates of moisture withdrawal and determining moisture penetration.

Soil moisture conditions in a Medford pear orchard during the 1939 season are shown in figure 2. The usable soil is 3 to 4 feet deep. Available soil

moisture, plotted in the upper set of curves, was determined by the standard oven-drying method. Each plotted point is the average of samples obtained at 10 locations uniformly distributed through the orchard. As each sample was taken, its stability was determined by the avallameter, and the average stability value of the 10 samples was plotted in the lower curves. Consequently, identical soil moisture conditions are shown in each chart, with the difference that units of available soil moisture were used in the upper and units of soil stability in the lower. In this orchard the soil stability at field capacity had previously been determined as 3.1, 4.1, and 5.5 pounds for 0-1-, 1-2-, and 2-3-foot depths, respectively. Stability corresponding to the wilting percentage for these depths was 44, 47, and 47 pounds, respectively. Thus the available range was established for each depth and these ranges are indicated on the chart, i.e., between 3.1 and 44 pounds for the 0-1-foot level, etc. Many observations of fruit growth rate in this orchard have shown that average soil moisture in the root zone must be maintained above 50 per cent available capacity for satisfactory yields. This soil has a stability of 10.7, 13.0, and 15.0 pounds at the mid-range of available moisture in the 0-1-, 1-2-, and 2-3-foot levels. A mean stability value of approximately 13 pounds for the 0-3-foot average appears, therefore, to be a practical criterion of need for soil moisture replenishment. A soil stability of 4 pounds closely represents field capacity throughout the upper 3 feet of this orchard, and 45 pounds corresponds to the wilting percentage.

A comparison of the two charts in figure 2 will show the general similarity of the two types of curves. Poor moisture penetration into the 2-3-foot level following each irrigation and the trend of moisture withdrawal can be as easily determined in terms of soil stability as in available moisture.

The shape of the soil stability curves, with increasing curvature downward, in spite of the decreasing rate of moisture withdrawal shown by the available moisture curves, is easily explained. Soil stability increases logarithmically as moisture is withdrawn from the soil, and therefore the two types of measurement cannot be parallel. If, instead of the uniform scale shown, a logarithmic scale is used in plotting the stability measurements in the soil stability curves, these will be very similar in shape to the soil moisture curves in the upper chart.

It will also be noted that the curves for the different soil depths do not occupy the same relative positions in the two charts. This is due to the plotting of the upper set of curves in percentage available moisture for each foot on a uniform scale in spite of the fact that each soil depth has a somewhat different available range. If these upper curves had been plotted on a moisture percentage basis, they would occupy almost exactly the same relative positions as they do in the lower set. The available moisture range would then be shown for each foot depth in a way similar to that shown for the stability curves.

Using stability measurements only, the grower can establish the available moisture range for his particular soil without resorting to expensive laboratory

techniques. Soil at field capacity can usually be found, at least in the upper foot, following a good irrigation and after the excess moisture has drained away. The soil stability can then be easily measured with the availameter. A stability value ranging from 2 to 5 pounds, depending on the soil type, will usually be found. If, following irrigation, soil samples from lower depths have stability values materially higher than this, the indication is that penetration was poor, that additional moisture could have been stored in the soil by irrigation, and that improved soil management or irrigation practice may be in order. The grower can approximate the lower limit of available moisture or the wilting percentage of his soil in terms of stability by measuring the soil stability in an adjacent field or fence corner where weeds, plants, or trees have permanently wilted because of lack of available moisture. A small patch of sunflowers might be planted for this purpose. A stability of approximately 45 pounds has usually been found to correspond to the wilting percentage of the heavy clay soils in the Medford locality.

The available moisture or stability range of the soil, which may be between about 3 and 45 pounds for a heavy clay, or between about 5 and 60 pounds for a medium-textured soil, once determined, should remain constant. Stability measurements between these two extremes will form a basis for judging the need for moisture replenishment. The point of plant stress may be a matter of judgment with the irrigator unless he can obtain specific recommendations for his particular soil, but he can be sure that the soil stability should not be allowed closely to approach the value corresponding to the wilting percentage. With pears on heavy clay soils he should not allow stability to exceed 12 to 15 pounds if he is interested in maximum sizes and yields. Once this stress point has been determined, either by experiment or by individual grower observation, a criterion on which to base irrigation practice is established. A few quick determinations on samples drawn from the important zones of the rooting system at periodic intervals will give an average soil stability figure which may be used in maintaining a simple chart similar to that of figure 2. Dates of impending soil moisture deficiencies can be accurately forecast from such a chart at least several days in advance, thus enabling better coordination of other orchard operations with irrigation. Such a graphic record is valuable in picturing soil moisture conditions in each of the root zone levels and gives the irrigator a guide toward more efficient irrigation practice.

Methods of calibrating and using the availameter for quantitative studies of soil moisture and irrigation practice have been worked out, and these together with basic data will be presented in the second part of this paper in an early issue of *Soil Science*.

#### SUMMARY

Orchard irrigation studies at Medford have shown knowledge of soil moisture conditions to be important in the timing of irrigation. A device called the "availameter" has been developed for use by orchardists and others in making



direct field soil moisture determinations without expensive laboratory equipment. This instrument measures the plasticity or *stability* of a soil core obtained with the soil tube. A close relationship between this measurement and the corresponding moisture content has been found.

The use of soil stability measurement as a direct measure of available soil moisture is shown. A simple method of determining the available soil moisture range and soil moisture available to plant use at any time is also presented.

The avallameter was not tried on light soils, but it is not considered adaptable to soils which fail to give cohesive soil tube cores; therefore, its use may be limited to medium or heavy soil types.

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## PLATE 1

A SOIL STABILITY DETERMINATION BEING MADE WITH THE AVAILAMETER

Note Details of the Sample Holder in the Foreground



1

2

3

4

# ORGANIC PHOSPHORUS IN SOILS: III. THE DECOMPOSITION OF SOME ORGANIC PHOSPHORUS COMPOUNDS IN SOIL CULTURES<sup>1</sup>

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In the general introduction to this series of papers (2), it was observed that our knowledge of the fate of individual organic phosphorus compounds introduced into soil is very deficient. It was felt that information of this kind would throw more light on the nature and significance of the organic phosphorus existing in soil than is afforded by results observed in cultures from which soil is absent. Experiments have therefore been designed with the object of tracing by chemical means the decomposition of nucleic acid compounds and phytin in the medium of the soil itself. The assumption underlying this work is that the dephosphorylation of an organic phosphorus compound produces a corresponding increase in the soluble phosphate of the soil. Since this approach to the problem seems to be in some respects a new departure in soil chemistry, the individual experiments are fully described in the following section.

## EXPERIMENTAL

Preliminary tests showed that soils treated with nucleic acid or phytin could not be dried by heat without producing a large increase in the soluble phosphate content. Consequently all analyses have been made on extracts of air-dried samples and are expressed on the moisture-free basis.

The general procedure followed was to incubate phosphorus compounds with 50-gm. portions of soil in loosely covered glass tumblers, which were stored in a dark room at  $23^{\circ}\text{C.} \pm 1^{\circ}\text{C.}$  The phosphatic material was ground up with a few grams of soil, and this intimate mixture was thoroughly incorporated with the remainder of the soil sample. Water was added to the cultures to provide a moisture content approaching the field moisture capacity, and more was added occasionally to replace that lost by evaporation. From time to time samples were withdrawn, air-dried, and analyzed for soluble phosphate.

<sup>1</sup> Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Quebec, Canada. Macdonald College Journal Series No. 151. Taken in part from a thesis by W. J. Dyer presented in partial fulfilment of the requirements for the degree of doctor of philosophy.

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*First decomposition experiment*

As a preliminary trial, ribonucleic acid (Eastman), "nucleotide" material separated without the use of oxalate from Ste. Clothilde muck soil, and pure  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  were incubated with portions of Ste. Clothilde muck soil. Samples were taken on mixing and after 45 days of incubation. Soluble phosphate was determined by photoelectric colorimetry in two successive extracts made with dilute sulfuric acid at pH 3.0 (6). The results obtained are summarized in table 1.

The apparent percentage decomposition was calculated from the increase in soluble phosphate, corrected for the relatively small decrease observed in the untreated sample. These decomposition figures are not accurate, since complete recovery of added inorganic phosphate was not obtained. It is evident, however, that the nucleic acid was decomposed to a large extent, whereas the soil product was relatively stable.

TABLE 1  
*Release of organic phosphorus in muck soil cultures*

MATERIAL ADDED	P ADDED	SOLUBLE P		APPARENT DECOMPOSITION
		At start	After 45 days	
	mgm.	mgm.	mgm.	per cent
None.....	0	3.54	2.55	0.0
Nucleic acid.....	248	4.43	161.20	63.8
Soil "nucleotide".....	100	5.90	11.35	6.5
$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ .....	200	142.60	113.00	-14.7*

\* The recovery of added phosphate was 70 per cent at the start and 55 per cent after 45 days.

*Second decomposition experiment*

In view of the evidence that part of the organic phosphorus of soil consists of nucleic acid derivatives, it was thought possible that the individual nucleotides would show marked differences in stability when incubated with soil. Accordingly, the four nucleotides of ribonucleic acid were prepared by the method of Levene and Bass (4), and their decomposition in soil was studied.

The soil used in this experiment was Macdonald College loam, a slightly calcareous fertile soil that is relatively poor in organic phosphorus. The individual cultures contained the following added materials: 1. None; 2. Ribonucleic acid; 3. Soil "nucleotide", the same product as was used in the previous experiment; 4. Guanylic acid; 5. Adenylic acid; 6. Cytidylic acid; 7. Caridylate; 8.  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ ; 9. Manure; 10. None. The details of the experimental procedure were the same as those in the first experiment. In table 2 the results are expressed in terms of the percentage release of added organic phosphorus observed after periods of 1 to 12 weeks. The manner in which these results were calculated will be evident from table 3, in which a complete set of extraction data is recorded.

Nucleic acid and the nucleotides were rapidly dephosphorylated, at least 70-80 per cent of their phosphorus being released within 1 week of incubation. There seems to be no significant difference between the nucleotides in this regard. The soil product again showed a high degree of stability. The organic phosphorus of manure was released slowly, but apparently progressively,

TABLE 2

*Release of soluble inorganic phosphorus, expressed as per cent of the organic phosphorus added*

TREATMENT	CULTURE NUMBER	SOLUBLE INORGANIC P				
		1 week	2 weeks	4 weeks	9 weeks	12 weeks
Yeast nucleic acid.....	2	80.26	88.66	....	....	67.36
Soil nucleotide.....	3	6.92	5.87	7.63	....	6.32
Guanylic acid.....	4	69.25	65.95	....	....	44.61
Adenylic acid.....	5	68.80	69.75	....	....	50.84
Cytidylic acid.....	6	73.82	72.00	....	....	46.96
Ca-uridyate.....	7	80.95	77.45	....	....	63.50
Manure.....	9	11.50	....	....	35.95	....
Inorganic phosphate*	8	85.12	80.72	....	....	68.00

\* Figures in this row show recovery of inorganic phosphate added.

TABLE 3

*Detailed results of extraction with 0.4 N H<sub>2</sub>SO<sub>4</sub>*

Weights per 100 gm. of soil

TREATMENT	CULTURE NUMBER	PHOSPHATE EXTRACTED	INCREASE OVER NIL (A)	INORGANIC P ADDED (B)	ORGANIC P RECOVERED AS INORGANIC (A - B)	ORGANIC P ADDED	RECOVERY OF ORGANIC P ADDED
		mgm. P	mgm. P	mgm.	mgm.	mgm.	per cent
None.....	1	76.0	....	....	....	....	....
Yeast nucleic acid.....	2	139.2	63.2	0	63.2	50.0	126.4
Soil nucleotide.....	3	90.8	14.8	1.56	13.2	48.44	27.3
Guanylic acid.....	4	123.1	47.1	12.20	34.9	37.8	92.4
Adenylic acid.....	5	130.7	54.7	6.60	48.1	43.4	110.9
Cytidylic acid.....	6	129.8	53.8	15.10	38.7	34.9	110.9
Ca-uridyate.....	7	124.7	48.7	2.78	45.9	47.22	97.3
Inorganic phosphate*....	8	125.8	49.8	50.0	-0.2	0	99.6

\* Figures in this row show recovery of inorganic phosphate added.

in contrast to the other materials. The significance of this behavior is obscure, as the identity of the phosphorus compounds in manure is unknown.

The recovery of added inorganic phosphorus was incomplete, and decreased from 85 per cent after 1 week to less than 70 per cent after 12 weeks. This could be due to reversion of the inorganic phosphate to less soluble forms, or to conversion into organic forms through assimilation and synthesis by micro-organisms. The latter process has been observed by Stoklasa (5) and termed



"biological absorption of phosphorus." If the decrease was due to reversion, it should be possible to recover the inorganic phosphate by extraction with a stronger acid. Accordingly, samples taken after 12 weeks were extracted twice with 0.4 *N* H<sub>2</sub>SO<sub>4</sub> solution, instead of with the dilute solution previously used. The results are given in table 3.

TABLE 4  
*Decomposition of organic phosphorus in two soil types*  
Weights per 100 gm. of soil

TREATMENT	SOLUBLE PHOSPHATE			APPARENT DECOMPOSITION OF ORGANIC P ADDED	
	0 weeks	1 week	3 weeks	1 week	3 weeks
	mgm. P	mgm. P	mgm. P	per cent	per cent
Macdonald College soil:					
1. Nil.....	77.57	79.74	77.40	0.0	0.0
2. Nucleic acid.....	81.08	112.74	115.20	58.98	68.58
3. Soil product.....	78.85	78.26	78.40	-5.52	-0.56
Podzol soil:					
4. Nil.....	12.17	12.20	11.86	0.0	0.0
5. Nucleic acid.....	12.26	15.64	17.03	6.70	10.16
6. Soil product.....	12.38	12.51	12.21	0.20	0.28

TABLE 5  
*Decomposition of phytin in soil cultures*  
Weights per 100 gm. of soil

TREATMENT	PHOSPHATE EXTRACTED			APPARENT DECOMPOSITION OF ORGANIC P ADDED	
	0 weeks	2 weeks	8 weeks	2 weeks	8 weeks
	mgm. P	mgm. P	mgm. P	per cent	per cent
Macdonald College soil:					
Nil.....	77.5	73.1	75.1	...	....
Phytin.....	78.1	74.3	101.9	1.2	50.4
Podzol soil:					
Nil.....	14.1	12.9	14.5	...	....
Phytin.....	13.7	13.9	16.8	2.8	5.4

The recovery of added inorganic phosphate was virtually complete. This shows that the net synthesis of organic phosphorus compounds was negligible. It appears that almost all the phosphorus of nucleic acid and the nucleotides was released as inorganic phosphate, although there are unaccountable variations in the results. The decomposition of the soil product still is seen to be relatively small.

*Third decomposition experiment*

The soil product prepared by the oxalate procedure (7) and ribonucleic acid were incubated with two soils, Macdonald College loam and a podzol from the Eastern Townships of Quebec, classified as Sherbrooke sandy loam. The details of procedure were the same as in the previous experiments, but 0.4 *N* H<sub>2</sub>SO<sub>4</sub> was used as the extractant. The results are given in table 4.

The dephosphorylation of nucleic acid proceeded vigorously in the Macdonald College soil, but relatively slowly in the podzol. The soil product appeared to be virtually unaffected in both soils.

*Fourth decomposition experiment*

Phytin, prepared from bran by the method of Boutwell (1), was incubated with the Macdonald College and podzol soils. The results are shown in table 5. Compared with nucleic acid, phytin was relatively stable in both soils. Decomposition was very slow in the podzol, but 50 per cent of the phytin phosphorus was released within 8 weeks in the Macdonald College soil.

## DISCUSSION

A method has been devised by which it is possible to trace the dephosphorylation of organic phosphorus compounds directly in the soil. The experiments so far performed have been of a tentative and exploratory nature, and it is not intended to attach strict quantitative significance to them. Certain relationships, however, are immediately evident.

The organic phosphorus preparations derived from soil are seen to be highly resistant to dephosphorylation by the soil microorganisms. Also there is evidence that inorganic phosphate added to soil is not rapidly built up into organic compounds but remains as inorganic phosphate. These results make it clear that the organic phosphorus of the soil represents an accumulation of stable forms and not a labile fraction maintained by synthetic activities. It appears to be the inactive end product of soil processes and hence relatively unavailable to both plants and microorganisms. This view does not preclude the likelihood that a small part of the organic phosphorus, such as that present in the tissue of microorganisms or recently added to the soil, is actively metabolized and readily available.

Ribonucleic acid and the four ribonucleotides, in contrast to the soil product, were rapidly dephosphorylated in soil. Two soils, however, differed widely in their power to decompose nucleic acid. The results which have been obtained do not show conclusively whether or not nucleic acids are completely broken down. The possibility exists that enzyme-stable residues are produced which contribute to the accumulation of organic phosphorus.

Phytin is relatively resistant to decomposition in soil, which indicates that it may be expected to accumulate as such under suitable soil conditions. Inositol phosphates other than phytin are undoubtedly formed during the

dephosphorylation of phytin, but at the moment there is no evidence to indicate whether these are more or less resistant than phytin itself.

The organic phosphorus of manure resembles phytin in its rate of dephosphorylation in soil cultures.

#### GENERAL DISCUSSION AND SUMMARY

In the introduction to this series of papers an attempt was made to define the state of knowledge concerning the organic phosphorus in soils and to indicate the principal problems awaiting solution. The investigations which have been reported throw some new light on these problems and warrant a further statement in definition of the nature and significance of organic phosphorus in soils.

The organic phosphorus occurring in soil represents an accumulation of compounds which for one reason or another are resistant to further decomposition. Because of this the organic phosphorus is a relatively unavailable fraction of the soil phosphorus. Its stability, however, like that of the soil organic matter as a whole, is to be regarded as relative rather than absolute, and doubtless it gradually becomes available through decomposition. Soil conditions influence the degree of accumulation to a considerable extent. Infertile, acid soils usually contain a high proportion of organic phosphorus, whereas neutral and calcareous soils have much less. The liming of acid soils might be expected to hasten the decomposition and so improve the phosphate status.

Phytin is one of the compounds that accumulate in soil. In acid soils it may enter into insoluble combination with sesquioxide constituents, in which form it is resistant to enzymatic hydrolysis. In neutral and calcareous soils it is probably more subject to decomposition, although still relatively resistant.

The partial dephosphorylation of phytin produces other phosphoric esters of inositol. There is no evidence regarding the relative stability of these compounds in the soil, but it is possible that they account for a significant proportion of the organic phosphorus, as Yoshida's results (8) seem to indicate.

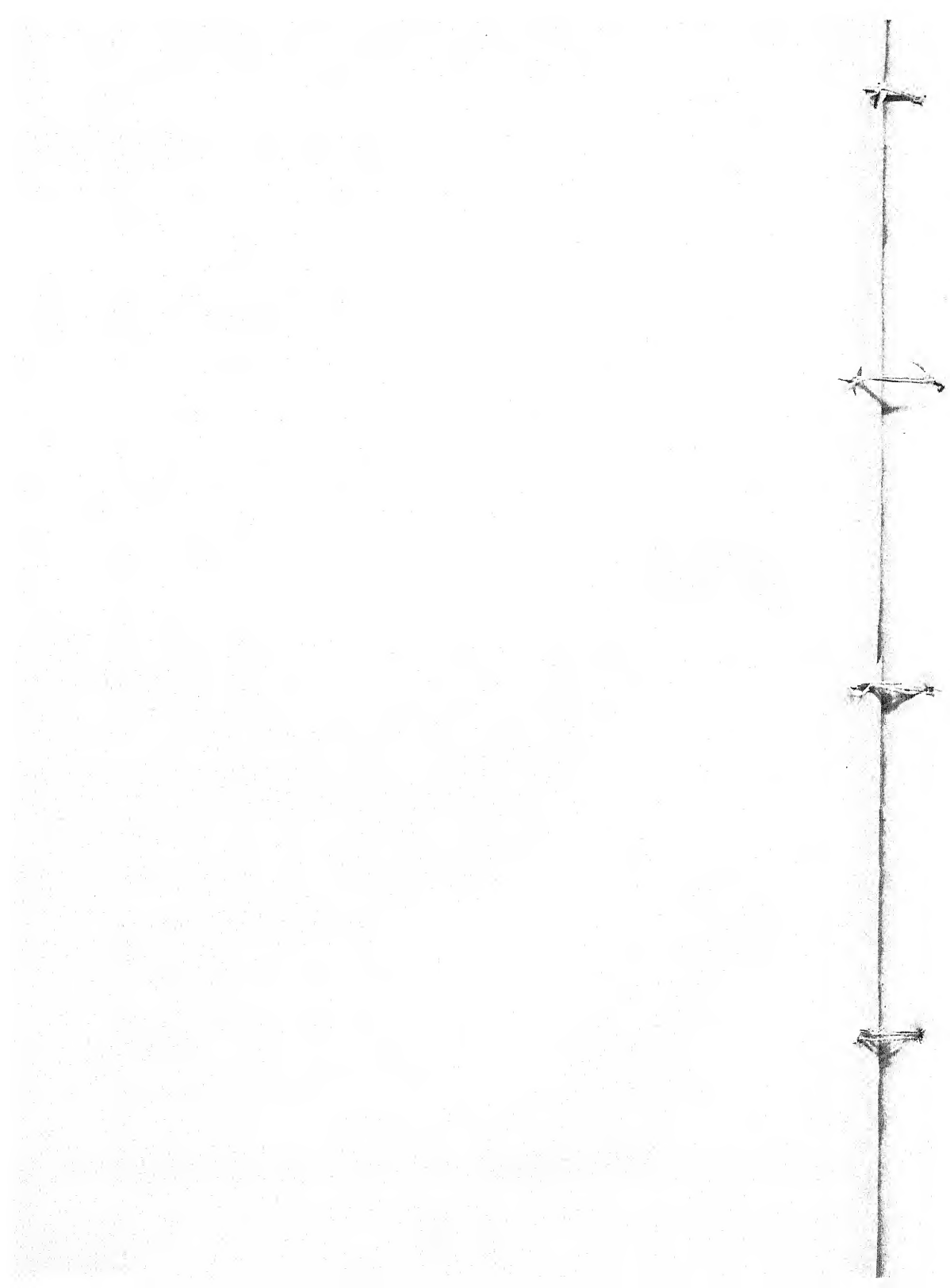
Nucleic acids have long been believed to make up a considerable proportion of the organic phosphorus in soils. It cannot be decided from the available evidence, however, whether or not this is actually so, although it remains highly probable. There is no doubt that nucleic acid derivatives are usually present in the preparations obtained from soils, but proof that these are combined with phosphorus is lacking. The purine constituents in the soil preparations are more difficult to release by acid hydrolysis than are the purines of ribonucleic acid. Moreover, ordinary ribonucleotides are readily dephosphorylated in soil cultures. If the nucleic acid derivatives in the soil preparations are nucleotides, therefore, their constitution must be different from that of ordinary nucleotides. Ribonucleic acid itself is readily dephosphorylated in soil cultures, but that it is completely broken down is not certain. The

observation of Gulland and Jackson (3), that 25 per cent of the phosphorus of ribonucleic acid is resistant to release by esterases, suggests the possibility that a nucleotidelike residue might accumulate in soil. Such a residue might be expected to remain after the partial decomposition of nucleic acid in organic materials added to the soil. Also, the protoplasm of microorganisms is rich in nucleic acids, which may be only partially broken down after the death of the organism. Each generation of the continuous succession of microorganisms living in the soil might thus make its contribution to a nucleotidelike residue, providing for a gradual accumulation of this form of organic phosphorus.

No one has yet been successful in identifying nearly all the organic phosphorus in any soil. The possibility exists, therefore, that other phosphorus compounds, not yet identified, are commonly present in soils, as Yoshida (8) was led to believe. The possibilities in this regard seem to be rather limited, however, unless it is postulated that a hitherto unknown type of phosphorus compound exists in soils. Lipoid phosphorus is present in the merest traces; sugar- and glycerol-phosphates are readily hydrolyzed by the nonspecific esterases which attack nucleotides; and the phosphorus of typical phosphoproteins, unlike the organic phosphorus of soils, is released by very mild alkaline hydrolysis. There is, therefore, some reason for thinking that virtually all the organic phosphorus of soil may be accounted for by the classes of compounds already designated; namely, phytin, inositol phosphates, and nucleotidelike substances.

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# THE NECESSITY FOR DIRECT CONTACT IN SULFUR OXIDATION BY THIOBACILLUS THIOOXIDANS

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The autotrophic, sulfur-oxidizing bacterium, *Thiobacillus thiooxidans*, presents many physiological problems, but none is more pertinent than a knowledge of the nature of the "mechanism by which insoluble elementary sulfur outside the cell is rendered soluble and can thus diffuse into the interior of the cell" (3). Since it is probable that a cell can derive energy from oxidations carried on only within its boundaries, and since, to enter into a cell, oxidizable materials must be soluble, the means by which sulfur oxidation might be achieved have remained matters for speculation.

The review of Bunker (3) makes extensive reference to the literature unnecessary. Suffice it to say that there are three hypotheses which seek to explain the ability of *Th. thiooxidans* to oxidize insoluble sulfur. These are:

1. The organism reduces the sulfur to hydrogen sulfide, which enters the cell and is reoxidized to sulfuric acid (2, p. 197 ff.; 7).
2. The sulfur is oxidized to soluble thionic acids by means of extracellular enzymes (2, p. 197 ff.; 3).
3. The sulfur is "hydrated" to soluble sulfur hydrates which can then enter the cell. A somewhat similar concept is that sulfur is soluble enough (or that its vapor pressure is high enough) for the organism's need (2, p. 197 ff.; 7).

All these hypotheses assume that the sulfur must be rendered soluble in the medium and disagree only as to the method by which it is converted into soluble materials. The evidence presented in this paper shows that it is not necessary for the sulfur to dissolve in the medium, but rather that the bacteria must be in direct contact with the sulfur particle before oxidation takes place.

## METHODS

The medium employed was that of Waksman (5), with slight modifications. The base medium ( $\text{NH}_4\text{Cl}$ , 0.3 gm.;  $\text{KH}_2\text{PO}_4$ , 3.0 gm.;  $\text{CaCl}_2$ , 0.25 gm.;  $\text{MgSO}_4$ , 0.5 gm.;  $\text{FeSO}_4$ , 0.01 gm.; to 1000 ml. with distilled water; initial pH adjusted to 4.8–5.0) was autoclaved 1 hour at 15 pounds steam pressure. Sulfur was sterilized separately by heating in boiling water for 3 hours and added to the

<sup>1</sup> This work was supported in part by the Wisconsin Alumni Research Foundation. A brief summary was presented before the Society of American Bacteriologists, St. Louis, December, 1940.

sterile medium under aseptic conditions. The autoclaved medium maintains a more favorable pH for a longer time than does a comparable steamed medium. Uninoculated controls may be kept for at least 4 weeks without change in sulfate content. The pH was determined with a glass electrode. Sulfate was determined by a turbidimetric method, which consists of precipitating 1 ml. of the sample with 1 ml. of 10 per cent  $\text{BaCl}_2$  in 0.1 *N* HCl and diluting the precipitate to 10 ml. with distilled water. Turbidity was measured with an Evelyn photoelectric colorimeter. By employing a large excess of barium chloride a precipitate of fine particles is obtained which gives reproducible turbidity. The method may be used over the range of 100 to 2,000 micrograms of sulfate-sulfur per milliliter with a precision of  $\pm 10$  micrograms. The organism used was a pure culture of *Thiobacillus thiooxidans*, Waksman and Joffe.<sup>2</sup> It was maintained on both liquid and solid media and checked for purity before use.

#### ORIGIN OF THE HYPOTHESIS OF DIRECT CONTACT

Among the controlling factors in the growth of *Th. thiooxidans* noted in the literature are oxygen and carbon dioxide. With respect to oxygen, two points have been particularly emphasized: First, if during sterilization of the medium, the sulfur should melt and settle to the bottom, sulfate formation is markedly decreased. Second, if equal quantities of sulfur and medium are placed in containers of different diameter, the rate of sulfate formation is greatest in the containers having the greatest diameter. Both of these effects have been interpreted as an alteration in the oxygen exchange (3, 4, 8). It was therefore thought possible to increase the growth rate by increasing the oxygen tension and by providing additional carbon dioxide; or, failing that, at least to decrease the rate of growth by decreasing the oxygen pressure. Results of experiments employing 10, 20, and 30 per cent oxygen and 0.01 to 10 per cent carbon dioxide are illustrated in figure 1. The data were obtained from cultures in large test tubes prepared under sterile conditions and inoculated with a pure culture of *Th. thiooxidans*. These tubes were placed in 2-liter flint glass bottles fitted with gas-tight stoppers. The gas mixture in each bottle was prepared by evacuation and by addition of the requisite quantity of the desired gas. In those cases where the total gases did not equal one atmosphere (10 per cent oxygen series) the remaining space was filled with pure hydrogen. Samples of the culture medium were removed periodically for analysis and the gases were replaced at each analysis.

In the data shown in figure 1, a small amount of variation is evident, but that variation bears no consistent relation to either oxygen or carbon dioxide pressure. Significantly, the oxygen can be decreased to half its value in air without detectable influence upon the rate of sulfate formation. This is not to say that oxygen and carbon dioxide have no effect upon sulfate forma-

<sup>2</sup> Obtained from S. A. Waksman, to whom we are indebted for this favor.

tion, since that is a matter for further study; but the experiments do illustrate that *Th. thiooxidans* is not sensitive to slight changes in oxygen tension, as had been supposed from previous experiments.

It seemed probable that throughout the experiments described there was some factor which remained constant and which was the controlling factor in sulfate formation. This factor could not be oxygen or carbon dioxide, since these were varied. However, in these experiments the sulfur-medium interface remained constant throughout the treatments, whereas in experiments employing different types of containers that interface had varied. The constancy of the results in our experiments and the relationship to diameter of

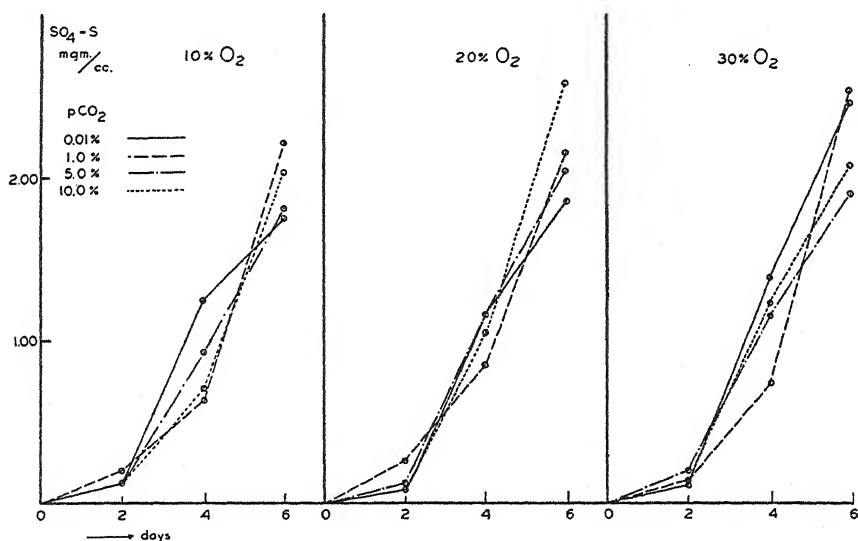


FIG. 1. INFLUENCE OF OXYGEN AND CARBON DIOXIDE TENSION UPON THE RATE OF SULFATE FORMATION BY PURE CULTURES OF *Thiobacillus thiooxidans*

container in the experiments of others could be explained if the controlling factor were the sulfur-medium interface rather than the oxygen or carbon dioxide relationships. Since the area of contact between sulfur and medium would have little influence upon the solubility of sulfur or upon its availability for external enzymatic activity, it appeared as though the bacteria were in direct contact with the sulfur particle. This concept would explain many of the results recorded in the literature. The older conceptions, which regarded the sulfur as being transformed into products soluble in the medium, could be replaced by the hypothesis that: *Th. thiooxidans must be in direct physical contact with the sulfur particle before sulfur oxidation can take place. It is neither necessary nor probable that the organism is able to render sulfur soluble in the medium.* This hypothesis implies that there is a certain amount of orientation of the bacteria at the surface of the sulfur particle and regards the



direct contact of the cell and the sulfur as a reflection of at least two possibilities, either that the sulfur is oxidized at the cell surface, or that it is dissolved in some constituent of the cell membrane before oxidation. This paper is concerned with the proof that direct contact between bacteria and sulfur is a *necessary* part of the means by which sulfur is oxidized but makes no attempt to explain the mechanism of sulfur oxidation beyond this point.

#### PROOF OF THE DIRECT CONTACT HYPOTHESIS

The correctness of the hypothesis may be tested in several ways. Five methods of approach are briefly recorded in the following paragraphs. Individually, they are explainable by the necessity for direct contact. Taken as a whole, we are able to find no other theory which will satisfactorily explain the experimental results.

##### *Collodion sac experiments*

One could confine either the bacteria or the sulfur within collodion sacs and thus prevent direct contact. Collodion sacs containing sulfur were suspended at the surface of the base medium and sterilized in flowing steam for 1 hour on 3 successive days. In some cases the sacs were empty and the sulfur was placed on the surface of the medium. Even after months of incubation there was no sulfate formation when the inoculum (1 ml. of an active culture of *Th. thiooxidans*) was placed on the opposite side of the collodion sac from the sulfur, yet active growth and sulfate formation took place within a week when the inoculum and sulfur were placed in direct contact. Similar experiments were made, using standardized cellophane dialyzing membranes, with the same results. It was only when the sulfur and the bacteria could be in direct contact that sulfate formation took place.

##### *Influence of alteration in sulfur-medium interface*

Another test of the direct contact hypothesis is the alteration of the sulfur-medium interface without change of any of the other conditions of growth. If this interface were altered there should be more room for bacteria-sulfur contact, and more cells could develop, with a consequent increase in rate of sulfate formation. Experimentally, two large porcelain-enameled trays were employed, each containing 1 liter of medium. This medium was autoclaved in 2-liter quantities, cooled, inoculated with 2 ml. of an active culture of *Th. thiooxidans*, and divided equally between the two trays. To each tray was added 2.5 gm. of sterile powdered sulfur. In one, it was spread over the entire surface of 1080 sq. cm.; in the other, it was confined to an area of about 56 sq. cm. by means of a glass ring suspended at the surface of the medium. Sulfate was determined periodically. In this experiment the surface of the medium exposed to air, its amount, the depth, the quantity of sulfur, the oxygen availability, and similar factors were constant. The only difference was in the area of the sulfur-medium contact. As illustrated in figure 2, the

tray having the greatest sulfur-medium contact shows the greatest rate of sulfate formation. Experiments employing the same principles with the additional variation of oxygen tension show differences (fig. 2) which were correlated with the area of the surface contact and not with the oxygen content.

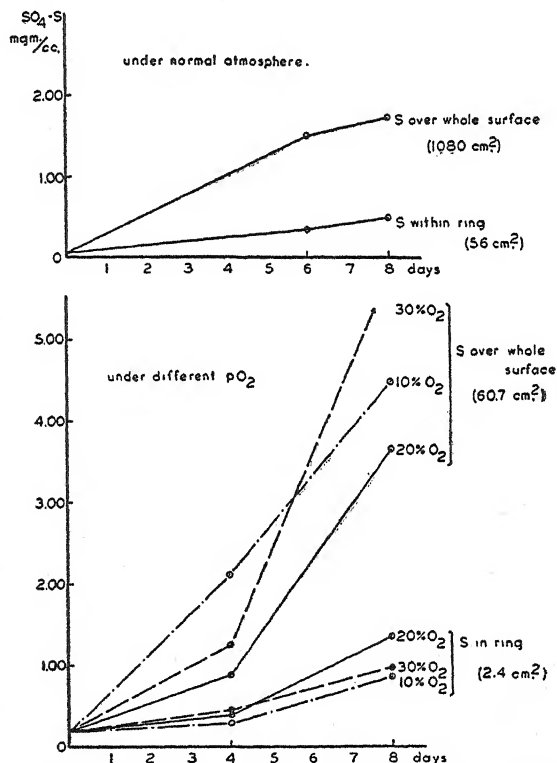


FIG. 2. INFLUENCE OF SULFUR-MEDIUM CONTACT UPON SULFATE FORMATION BY *Thiobacillus thiooxidans*

### Microscopic observations

If direct contact between sulfur and bacteria is necessary for sulfur oxidation, such contact should be visible with the microscope. Indeed, Waksman (8) has noted that a particle of sulfur from a growing culture of *Th. thiooxidans* is surrounded by bacteria, and observations of slides prepared from surface films show the bacteria clustered about the sulfur granules. From these studies there is considerable evidence of an orientation of the bacteria at the surface of the sulfur particle. Microscopic counts of growing cultures show populations ranging from 2 million to 15 million per milliliter at the surface with approximately one-third that number at depths only 1 inch below. At any point below the surface the population is always markedly less than at the surface layer. The turbidity noted in the medium results not from growth

throughout the medium but from organisms settling from the surface or moving through the medium in search of sulfur particles. Considerably more data are available from microscopic observations, but those cited provide sufficient evidence in support of the direct contact concept.

#### *Effect of surface agents*

Additions of materials such as ground glass or charcoal would not be expected to alter bacteria-sulfur contact if these were submerged, but might have an effect if they could be kept at the surface. Though Ayyar (1), with an organism related to, but apparently not identical with, *Th. thiooxidans*, has reported marked increases in the rate of sulfur oxidation by such additions, we are unable to find any marked effect upon *Th. thiooxidans*. Decreases in sulfate production have been observed with glass wool and glass beads when these were not entirely submerged, and increases may sometimes be obtained if the material tends to buffer the medium at a more favorable pH.

If the surface tension of the medium is altered, either with oils or with neutral wetting agents (such as aerosol), the rate of sulfate formation is frequently increased. In a medium prepared with 0.001 per cent aerosol, the sulfur will not remain at the surface but will rapidly settle to the bottom. At the bottom its rate of oxidation is often greater than at the surface. Mineral oil in small quantity has no particular effect, although if added in quantity ( $\frac{1}{4}$  inch layer over the surface) it may decrease sulfate formation slightly. Experiments employing wetting agents are not entirely consistent, since the actual conditions at the sulfur-bacteria interface are difficult to reproduce.

#### *Influence of size of sulfur particle*

It would be expected that the smaller the sulfur particle, the greater would be the surface exposed to the bacteria per unit weight of sulfur and thus the greater the rate of sulfate formation, other conditions being equal. If one compares sulfur passing through sieves of 200 mesh per square inch with droplets of melted sulfur, the powdered form produces 4-5 times as much sulfate per unit time. Similar results have been reported (6). One would expect a limit to be reached, however, beyond which further decrease in the size of the sulfur particle would have no essential effect. Though the total surface exposed by the sulfur increases rapidly with each division, the surface available for bacterial contact increases much less rapidly, and conditions of competition, of actual food supply in the particle, and space considerations might then become limiting factors. Under the conditions of our experiments (1 gm. sulfur; 100 ml. base medium; in 250-ml. flasks) there is a progressive increase in the rate of sulfate formation until a definite point is reached. Sulfur particles passing through sieves of 20 mesh (*or less*) per square inch yielded identical rates of sulfate formation, whereas sizes larger than these gave progressively increasing rates with decrease in particle size.

## SUMMARY AND CONCLUSIONS

On the basis of results reported in this paper it is concluded that *Th. thiooxidans* must be in direct physical contact with the sulfur particle before sulfur oxidation can take place. It is neither necessary nor probable that the organism can render sulfur soluble in the medium. The autotrophic *Th. thiooxidans* is shown to be in accord with the generalization that no cell can obtain energy from oxidations which are not carried out at or within its boundaries.

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# THE EFFECT OF CERTAIN SOIL FACTORS ON THE YIELD OF WHEAT IN THE PUNJAB

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The soils of the Punjab plains which do not give a normal crop yield are generally highly saline or alkaline. The salt present in the soil may, under certain conditions, accumulate on the surface to such an extent as to render the soil uncultivable. When the pH value of the soil becomes greater than 8.5, the yield of crops diminishes markedly. It has been shown elsewhere (10) that for a normal yield of rice the soluble salts must not exceed 0.24 per cent and the pH value, 8.8.

It is known that the deterioration of the soils of the Punjab is accompanied by an increase in the proportion of sodium to calcium in the exchange complex (15). Besides the major elements such as Na, K, Ca, and Mg, however, there are others which, although occurring in traces, are known to exercise a profound influence on crop production. Of these trace elements manganese, boron, and iron are considered to be most important (2, 6) and are, in fact, essential for normal growth. The preliminary experiments revealed a qualitative relationship between the fertility and the manganese content of the soil; i.e., poor soils contained a higher percentage of manganese than did soils of good quality. Some soils contained as much as 5.6 m.e. of manganese. This observation was of sufficient interest to justify a more thorough investigation with the object of discovering other factors that might affect the crop yield on such soils.

As wheat is the major agricultural crop of the province, its yield was taken as the basis of comparison for purposes of this study. At each of more than 200 sampling sites selected in eight districts of the province, the yield of wheat during the year was ascertained, and samples of the top and of the second 9 inches of soil were collected. A systematic examination of a number of factors which might influence the yield of wheat was made. The results of the various analyses of the soils also were examined statistically to determine whether any significant correlation existed between the yield figures and some of the characteristics of soils determined.

The results of the various analyses of the soils and their correlations with wheat yields are presented in this paper.

## EXPERIMENTAL

The soils were analyzed for total soluble salts; pH; exchangeable Na, K, Ca, and Mg; total manganese; available phosphates; and boron. The total

nitrogen and iron contents of a few samples were also determined to gather information on the mutual relationship between manganese and nitrogen or iron in soils, as it is known that the action of manganese in the plant is linked with that of nitrogen (13, p. 104), and injury to pineapples has been attributed to interference by manganese with the iron in the plants (12).

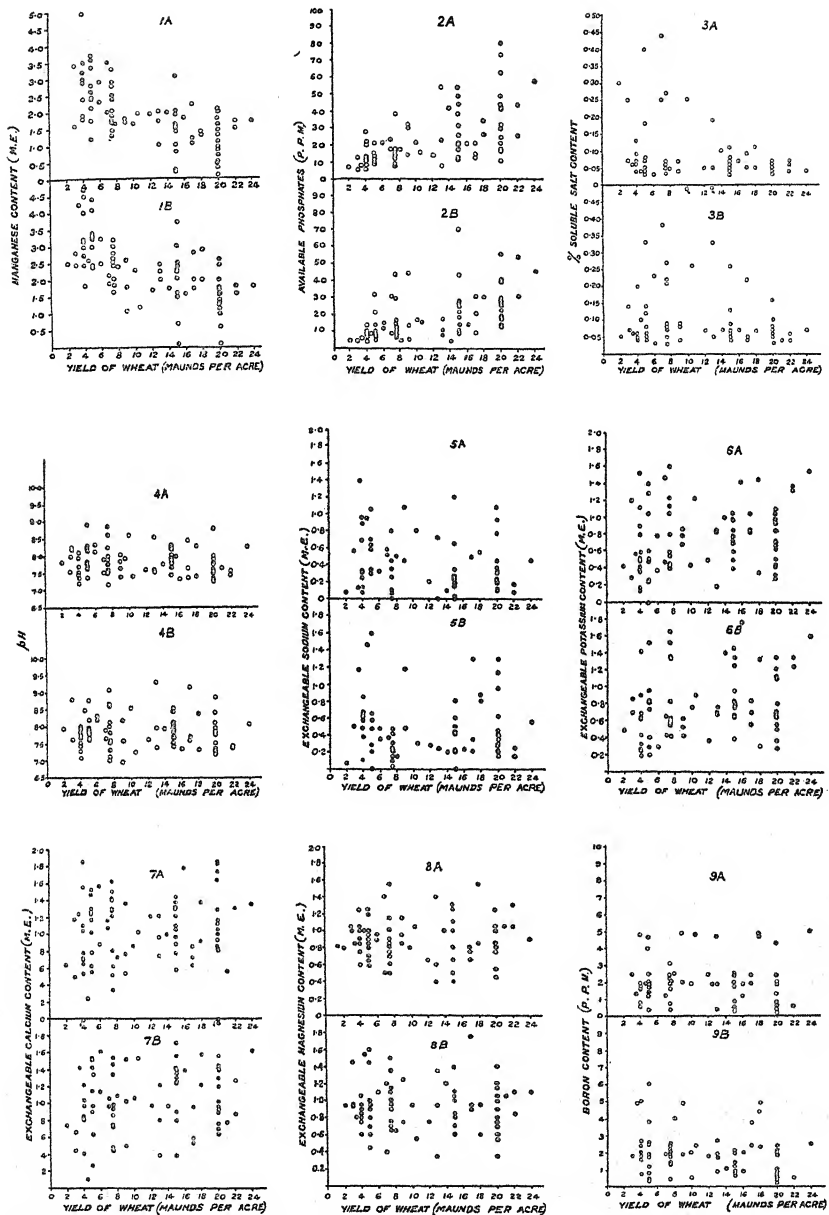
The following is a brief description of the technique adopted for the various analyses:

The pH values were determined with the glass electrode using a 1:5 soil: water ratio (5). The manganese was determined by the bismuthate method (7). The phosphates were extracted from the soils with  $\text{CO}_2$  (11), and the Denigès technique as elaborated by Chapman (3) was used to develop color. The colorimetric comparison was done with a Bolton and Williams photo-electric colorimeter against colors developed with phosphate solutions of known strengths. Boron was extracted with hot water and determined colorimetrically with quinalizarin (14). Nitrogen was determined by Kjeldahl's method as modified by Bal (1). Various buffer mixtures were tried for leaching iron from the soils. Best results were obtained, however, with the buffer solution containing 1.02 per cent potassium hydrogen phthalate and 0.13 per cent HCl. Total iron was determined in an aliquot of the leachate with thioglycolic acid, and ferric iron was determined in another portion of the leachate with "ferro" (18). Exchangeable bases and total soluble salts were determined by the usual methods.

In figures 1-11, the results of various analyses have been plotted separately for the top and the second 9-inch soil samples against the yield of wheat (expressed in maunds, each of which is equivalent to 82 pounds, per acre). In figure 12, the manganese contents of soils have been plotted against the nitrogen contents.

#### *Statistical treatment of analytical results*

Little work has heretofore been done in the Punjab to correlate the yield of wheat with the different soil characteristics, especially with the trace elements present in the soils. At Rothamsted, Fisher (4) has studied the influence of rainfall, and Tippet (16), the influence of sunshine on the yield of wheat. In India, Unaker (17) has worked out the correlation between weather and crops with special reference to the Punjab wheat, and Kalamkar and co-workers (8, 9) have investigated the effect of weather and prices on the cotton acreage and yield per acre in the Bombay Presidency. Most of the investigators, however, treat the yields over a number of years. The present investigation is different in that it takes individual fields in different districts and considers the yield of wheat at the time of sampling, i.e., during one year only. Statistical analyses, the results of which are given in table 1, have been made for each of six districts separately in order to bring out the extent of variation of the correlation coefficients for various soil characteristics. The



FIGS. 1-9. Yield of Wheat in Relation to: 1, Manganese Content; 2, Available Phosphate Content; 3, Soluble Salt Content; 4, pH; 5, Exchangeable Sodium Content; 6, Exchangeable Potassium Content; 7, Exchangeable Calcium Content; 8, Exchangeable Magnesium Content; and 9, Boron Content, of (A) the Top and (B) the Second 9-inch Soil Samples



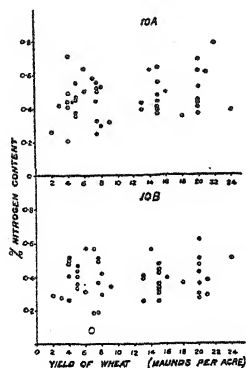


FIG. 10. YIELD OF WHEAT IN RELATION TO THE NITROGEN CONTENT OF (A) THE TOP AND (B) THE SECOND 9-INCH SOIL SAMPLES

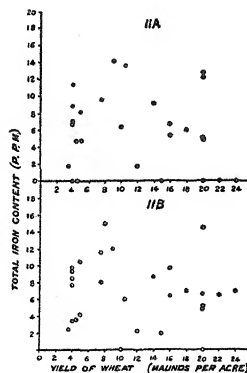


FIG. 11. YIELD OF WHEAT IN RELATION TO THE TOTAL IRON CONTENT OF (A) THE TOP AND (B) THE SECOND 9-INCH SOIL SAMPLES

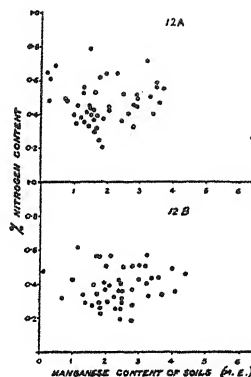


FIG. 12. RELATION BETWEEN THE MANGANESE AND THE NITROGEN CONTENTS OF (A) THE TOP AND (B) THE SECOND 9-INCH SOIL SAMPLES

number of soils, the results of which formed the basis of any particular statistical examination, is indicated in column 3 of table 1.

The following are the notations used in the statistical interpretation of the analytical results:

Variable 1 = The yield of wheat in maunds per acre.

Variable 2 = Analytical results for the characteristic under consideration, e.g., manganese, phosphates, pH, etc., for the top 9-inch soil samples only.

Variable 3 = Analytical results for the characteristic under consideration for the second 9-inch soil samples only.

$r_{12}$  = Total correlation between variables 1 and 2.

$r_{13}$  = Total correlation between variables 1 and 3.

$r_{23}$  = Total correlation between variables 2 and 3.

$R$  = Multiple correlation of variable 1 with variables 2 and 3.

$A_{12}$  = Per cent variance of yield expressible in terms of variable 2 alone.

$A_{13}$  = Per cent variance of yield expressible in terms of variable 3 alone.

$A$  = Per cent variance of yield expressible in terms of variables 2 and 3 taken together.

### Discussion of results

*Correlation of manganese content of soils and the yield of wheat.* All the correlations of manganese content with yield are negative. They vary from  $-0.51$  to  $-0.69$  and from  $-0.38$  to  $-0.76$  in the top and second 9 inches of soil respectively. The mean values of the correlations of manganese contents in the top and second 9-inch soil samples with yield figures are nearly equal, i.e., about  $-0.58$ . On the average, about one-third of the variation in yield can be explained in terms of the manganese content of the soils. The multiple correlation improves significantly the percentage of variation of yield as related to the manganese content in the districts of Multan, Gujrat, Jhang, and

TABLE 1

*Correlation between wheat yield and certain soil characteristics for six of the districts of the Punjab*

SOIL CHARACTERISTIC	DISTRICT	NUMBER OF OBSERVATIONS	$r_{23}$	$r_{12}$	$r_{13}$	$R$	$A_{12}$	$A_{13}$	$A$
Manganese	Multan	12	.0467	-.5211	-.6733	.8329	19.86	39.86	62.56
	Gujranwala	15	.8016*	-.6102*	-.7564†	.7547†	32.41	53.92	49.57
	Gujrat	26	.0391	-.5408†	-.3804	.6494†	26.30	10.90	37.14
	Jhang	28	.4471*	-.5803†	-.5433†	.6613†	31.12	26.81	39.23
	Lyallpur	26	.7323*	-.6939†	-.7374†	.7685†	45.99	52.47	55.50
	Shahpur	37	.4288†	-.5074†	-.5291†	.6134†	23.63	25.94	33.96
Phosphates	Multan	12	.6755*	.4502	.6542*	.6542	12.30	37.08	30.09
	Gujranwala	17	.4410	.5834*	.4757	.6321*	29.64	17.47	31.38
	Gujrat	25	.5688†	.7248†	.4554*	.7266†	50.47	17.29	48.51
	Jhang	27	.8485†	.3937*	.5585†	.5784†	12.12	28.44	27.92
	Lyallpur	32	.2443	.4890†	.5344†	.6498†	21.38	26.18	38.23
	Shahpur	35	.5957†	.7103†	.6372†	.7587†	48.95	38.80	54.91
Total salt	Multan	12	.3571	-.4167	-.0911	.4212	9.10	.....	.....
	Gujranwala	17	.7344	-.1395	.0369	.2124	.....	.....	.....
	Gujrat	24	.8826	.1957	.1537	.1995	.....	.....	.....
	Jhang	24	.4000	.2127	-.0810	.2787	0.15	.....	.....
	Lyallpur	32	.8841	-.4361*	-.4070*	.4385	16.32	13.78	13.66
	Shahpur	36	.7143	-.3191	-.2335	.3192	7.54	2.67	4.75
pH	Multan	12	.6028	-.2663	-.4453	.4452	.....	11.81	2.00
	Gujranwala	16	.8811	-.1341	.0433	.2975	.....	.....	.....
	Gujrat	26	.3763	-.1171	.1632	.2524	.....	.....	.....
	Jhang	28	.9462	.1017	.0160	.2676	.....	.....	.....
	Lyallpur	31	.8301	-.1319	-.8480	.1389	.....	.....	.....
	Shahpur	37	.8516	.0502	.0186	.0663	.....	.....	.....
Sodium	Multan	12	.8522	-.5174	-.4669	.5202	19.44	13.98	10.85
	Gujranwala	16	.7906	-.1222	-.3296	.3998	.....	4.51	3.07
	Gujrat	23	.6430	-.1092	.0427	.1833	.....	.....	.....
	Jhang	23	.3151	-.3126	-.0141	.2886	3.37	.....	.....
	Lyallpur	33	.8735	.0193	.0038	.0331	.....	.....	.....
	Shahpur	36	.1282	.1319	.0068	.1323	.....	.....	.....
Potassium	Multan	12	.1505	.0605	.5414	.5418	.....	22.24	13.66
	Gujranwala	12	.9306†	.0561	.0850	.1058	.....	.....	.....
	Gujrat	24	.8955†	.1762	.2319	.2421	.....	1.07	.....
	Jhang	22	.7974†	-.1588	-.0277	.2285	.....	.....	.....
	Lyallpur	32	.6980†	-.1944	-.2016	.2149	.58	.87	.....
	Shahpur	34	.7256†	.7381†	.7122†	.7814	53.06	49.18	58.55

\* Significant at the 5 per cent point.

† Significant at the 1 per cent point.

TABLE 1—*Concluded*

SOIL CHARACTERISTIC	DISTRICT	NUMBER OF OBSERVATIONS	$r_{23}$	$r_{12}$	$r_{13}$	$R$	$A_{12}$	$A_{13}$	$A$
Calcium	Multan	12	.5400	-.0625	.1996	.2839	.....	.....	.....
	Gujranwala	17	.6703†	-.0890	-.1155	.1157	.....	.....	.....
	Gujrat	22	.8547†	.2185	.3351	.3592	0.013	7.79	3.75
	Jhang	22	.8556†	.4430	.3890	.4434	15.60	10.87	11.21
	Lyallpur	31	.6438†	.4584	.2699	.4594*	18.29	4.09	12.64
	Shahpur	32	.7053†	.1096	.1340	.1356	.....	.....	.....
Magnesium	Multan	12	-.0021	.1312	-.0595	.1438	.....	.....	.....
	Gujranwala	16	.6352	-.1631	-.0975	.1630	.....	.....	.....
	Gujrat	23	.4998	-.0137	.0203	.0331	.....	.....	.....
	Jhang	23	.5534	.0917	.5013	.5485	.....	21.57	23.10
	Lyallpur	33	.1245	-.0795	-.0334	.2607	.....	.....	.....
	Shahpur	36	.3389	.2012	.1901	.2397	0.19	0.78	0.04
Boron		74	.4960	-.0473	-.2823	.3018	.....	6.70	6.55
Nitrogen		50	.5899	.1835	.0730	.1886	1.36	.....	.....

NOTE:  $r_{23}$  = total correlation between the analytical results of the top and the second 9-inch soil samples.  $r_{12}$  = total correlation between the analytical results of the top 9-inch soil samples and the yield of wheat.  $r_{13}$  = total correlation between the analytical results of the second 9-inch soil samples and the yield of wheat.  $R$  = multiple correlation between the analytical results of the top and the second 9-inch soil samples, and the yield of wheat.  $A_{12}$  = percentage variance of yield expressible in terms of analytical results of top 9-inch soil samples.  $A_{13}$  = percentage variance of yield expressible in terms of analytical results of second 9-inch soil samples.  $A$  = percentage variance of yield expressible in terms of analytical results of top and second 9-inch soil samples taken together.

Where the values of  $A_{12}$ ,  $A_{13}$ , and  $A$  are not given, the calculations work out to be negative, which means that no relation exists between the variables concerned.

Shahpur, but there is no significant improvement in the case of Gujranwala and Lyallpur. There seems to be no significant correlation between the manganese content of the top and the second 9-inch samples in the districts of Multan and Gujrat, but in the districts of Gujranwala and Lyallpur the correlation is very high.

*Correlation of phosphate content of soils and the yield of wheat.* All the correlations of phosphate with yield are positive and vary from 0.39 to 0.72 and from 0.46 to 0.65 for the first and second 9-inch soil samples. The mean values of the correlations with both the top and the second 9-inch soil samples are nearly equal to 0.57. On an average, about one-third of the variation in yield can be explained in terms of the phosphate content. The multiple correlation improves significantly the percentage of variation of the yield in terms of phosphate in the Lyallpur District. In Shahpur, the difference though significant is not high. Other districts do not show any significant improvement. The correlations of the phosphate content of the first 9-inch samples with that

of the second 9-inch samples are insignificant for soils of the Lyallpur and the Gujranwala Districts. In the other districts the correlations are significant.

*Correlation of the total soluble salt content of soils and the yield of wheat.* No significant correlation between the total soluble salt content and the yield of wheat is obtained except in the Lyallpur District, where the correlation is negative. About one-eighth of the variation in yield in the Lyallpur District can be explained in terms of the total soluble salt content.

*Correlation of pH of soil and the yield of wheat.* All the correlations of pH of the top and second 9-inch soil samples with yield are low and insignificant.

*Correlation of the exchangeable sodium content of soils and the yield of wheat.* The correlations of sodium contents of the top and second 9-inch soil samples and the yields of wheat are insignificant.

*Correlation of the exchangeable potassium content of soils and the yield of wheat.* The only significant correlations of yield with potassium are in the Shahpur District. Both  $r_{12}$  and  $r_{13}$  for this district are positive and are nearly equal, having a value of about 0.72. Though high yield appears to be associated with high potassium content of the second 9-inch soil samples of the Multan District, the results cannot be accepted as definitely proved. In the other districts there seems to be no association at all between the potassium content of soils and the yield. About one-half of the variation in yield in the Shahpur District can be explained in terms of the potassium content of either the top or the second 9 inches of soil. The variation of yield explicable in terms of potassium content is improved slightly in the Shahpur District by the multiple correlation coefficient. All the correlations between the potassium contents of the top and second 9-inch soil samples are significant except that in the Multan District.

*Correlation of the exchangeable calcium content of soils and the yield of wheat.* The only significant correlations of yield and the calcium contents of soil are obtained with the top 9 inches in the Jhang and Lyallpur Districts, though even here they do not reach the 5 per cent level of significance. The two correlations are, however, nearly equal, having a value of about 0.45. The correlations for soils of other districts are low and insignificant. About one-sixth of the variation in yield in the districts of Jhang and Lyallpur can be explained in terms of the calcium contents of the top 9-inch soil samples. The correlations between the calcium contents of the top and second 9 inches of soils are, on the whole, significant. Although the correlation for Multan as judged by Fisher's test does not reach the 5 per cent point, it might be considered as significant in view of the correlations obtained for other districts.

*Correlation of exchangeable magnesium content of soils and the yield of wheat.* No correlation seems to exist between the magnesium content and the yield of wheat in the soils examined.

*Correlation of the boron content of soils and the yield of wheat.* Correlations involving the boron content of about 150 soil samples representing different

yields of wheat from the various districts of the Punjab were determined on the total number of samples and not by districts. The correlations between the boron content of the top and the second 9-inch soil samples and the yield figures are negative and are not significant.

*Correlation between the nitrogen content of soils and the yield of wheat.* About 50 sites from the various districts having different yield figures were selected and the nitrogen contents determined. The correlations between the nitrogen content of the top and the second 9-inch soil samples and the yield figures are positive but are not significant. There appears to be no marked relation between the nitrogen and manganese contents of the soils (fig. 12).

*Correlation between the total iron content of soils and the yield of wheat.* Total iron and ferric iron contents of a few selected soils were determined, but the values were so varying that it was not possible to work out any correlation between the total iron content of the soils and the yield figures.

*Mutual correlation coefficients between the manganese, phosphate, and potassium contents of the soils.* Very significant correlations have been shown to exist between the manganese and phosphate contents of soils and the yields of wheat. It was thought that the characteristics which gave significant correlations with yield figures might have a correlation between themselves. The mutual correlations of manganese, phosphates, and potassium content of the soils were therefore examined. The soils of the Shahpur and Lyallpur Districts were selected for this study because these two districts had the largest number of observations. The correlations already obtained for these districts were as follows:

DISTRICT	MANGANESE		PHOSPHATE		POTASSIUM	
	0-9 inches	9-18 inches	0-9 inches	9-18 inches	0-9 inches	9-18 inches
Shahpur.....	-.51	-.53	.71	.64	.74	.71
Lyallpur.....	-.69	-.74	.49	.53	-.19	-.20

The following mutual correlation coefficients were obtained:

DISTRICT	MANGANESE AND POTASSIUM		MANGANESE AND PHOSPHATE		PHOSPHATE AND POTASSIUM	
	0-9 inches	9-18 inches	0-9 inches	9-18 inches	0-9 inches	9-18 inches
Shahpur.....	-.23	-.40	-.35	-.35	0.54	0.55
Lyallpur.....	.15	.001	.47	-.31	-.37	-.28

The results show that:

In the Shahpur District all the correlations except that between the manganese and potassium contents of the top 9-inch soil samples are significant on the 5 per cent level. The correlations between the potassium and phosphate contents in both the top and the second 9-inch soil samples are significant

on the 1 per cent level. The results indicate that in this district a high phosphate content in the soil is usually associated with a high potassium content. Moreover, a high manganese content appears to be associated with low phosphate and potassium contents, but this must not be considered as fully established until further confirmation is available.

In the Lyallpur District no association between the manganese and potassium contents in the first or second 9-inch soil samples is apparent. The manganese and phosphate contents appear to be negatively correlated only in the subsurface 9 inches. The correlation for the first 9 inches is highly significant, whereas that for the second 9 inches is insignificant on the 5 per cent level. The potassium and phosphate contents also seem to be negatively correlated. The correlation for the top 9 inches is significant on the 5 per cent level, whereas that for the second 9 inches is insignificant.

As far as the mutual correlation of the phosphate and potassium contents is concerned the results obtained for the Shahpur District are the reverse of those of the Lyallpur District. It appears, therefore, that to obtain more definite information regarding the mutual correlations of various characteristics, an examination of a very large number of observations would be necessary.

*Mutual correlation between the nitrogen and manganese content of soils.* As described before, the action of manganese in the plant is linked with that of nitrogen (13, p. 104). The mutual correlation between the manganese and nitrogen contents of soils did not, however, reveal any significant relation between these two characteristics in the soils examined.

No mutual correlation between the total iron and manganese contents of soils could be obtained.

#### CONCLUSIONS

The examination of the analytical results by the method of correlational analysis has revealed that significant correlations are obtained between the yield of wheat and the manganese and available phosphate contents of soils. In general, soils giving a high yield of wheat have a low manganese and a high available phosphate content.

The effect of manganese cannot as yet be explained. The soils of the Punjab are generally alkaline, the range of pH values of wheat soils being from 7 to 8.5. No correlation is found between the yield of wheat and the pH value. Within this range of pH values manganese is insoluble; it seems, therefore, that manganese may not affect directly the yield of plants but some other factor determining the accumulation of manganese in the soils may be the real cause of relationship. The mutual correlations between the manganese content and the phosphate, potash, or nitrogen bring out the fact that the best correlation existed between manganese and phosphate, although the value is not very significant. It is proposed to continue the investigation in the laboratory and in the field.

## SUMMARY

A number of soil samples from the various districts of the Punjab have been examined for their contents of manganese; phosphates; total soluble salts; exchangeable Na, K, Ca, and Mg; boron; nitrogen; and iron and for their pH values. Statistical correlations between the yield and the various characteristics have also been worked out. It has been shown that a significant and negative correlation exists between the manganese content of soils and the yield of wheat and an equally significant and positive correlation exists between the phosphate content of soils and the yield. The correlations between the other characteristics examined and the yield figures are not very significant.

The mutual correlations between manganese and nitrogen or manganese and potassium are not significant. There seems to be a negative, though insignificant correlation, however, between the manganese and the phosphate content of soils.

As a result of this investigation the importance of manganese and phosphate contents of soils in relation to the yield of wheat in the Punjab is brought out.

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## THE BEHAVIOR OF SOLUBLE ORGANIC PHOSPHATES ADDED TO SOILS

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In a previous investigation (3) conducted at this laboratory, using glycerophosphate as a typical organic phosphate, it was observed that in Cecil clay loam, a soil of comparatively high colloid content, the phosphorus added as organic phosphate became water-insoluble about as quickly as in the case of added soluble inorganic phosphates. In the light-textured Norfolk sandy loam the phosphorus in glycerophosphate tended to remain in solution for several days longer than that in added monopotassium phosphate. In the calcareous Las Vegas loam this difference between the two phosphates was even more marked. In an unpublished study on the effect of temperature on the hydrolysis and retention of organic phosphates in soils it was found that glycerophosphate was converted into the inorganic form and fixed as such. In general, the organic phosphate behaved like monopotassium phosphate so far as solubility and retention are concerned except for an initial delay occurring while the organic form was being changed to the inorganic form. The rates of hydrolysis and retention of glycerophosphate were much greater at 35°C. than at 5°C., largely because of the greater activity of soil microorganisms. The data on monopotassium phosphate, so far as comparable, were in accord with those previously reported (3).

In the present studies the behavior of several other organic phosphates in soils of different textures and maintained under aerobic and anaerobic conditions is reported. These studies were planned in part to furnish information on the penetrability of these compounds in soils.

### MATERIALS AND METHODS

The soils used chiefly were Norfolk sandy loam, Las Vegas loam<sup>2</sup>, and Cecil clay loam, described previously (3). The reactions of these soils were pH 5.1, 8.4, and 4.8, respectively. In a few experiments Chester loam (A<sub>2</sub> horizon, 2 to 10 inches), obtained near Rockville, Md., was employed. This soil,

<sup>1</sup> The writers are indebted to G. E. Hilbert, K. D. Jacob, and L. T. Alexander for numerous suggestions; to G. E. Hilbert and N. J. Hipp for the preparation of some of the phosphate compounds; and to the Monsanto Chemical Company and Commercial Solvents Corporation for supplying certain other phosphates used.

<sup>2</sup> This soil is essentially free from alkali salts, contrary to a previous statement (3), but is rather calcareous. An analysis made by L. T. Alexander showed 750 p.p.m. of soluble salts.

according to Brown and Byers (1), has 31.6 per cent clay, 19.7 per cent colloid, 41.1 per cent silt, and a pH of 4.8.

The organic phosphates used and their purity were as follows:

Sodium glycerophosphate from Merck and Company. Analysis: calculated for  $\text{Na}_2\text{C}_3\text{H}_7\text{PO}_6 \cdot 5\text{H}_2\text{O}$ —P 10.13,  $\text{H}_2\text{O}$  29.42; found—P 10.67,  $\text{H}_2\text{O}$  26.49.

Calcium hexose diphosphate from Winthrop Chemical Company. Analysis: calculated for  $\text{Ca}_2\text{C}_6\text{H}_{10}\text{P}_2\text{O}_{12}$ —P 14.90; found—total P 14.14 and inorganic P 1.83 after 6.12 per cent loss in moisture following drying in an Abderhalden apparatus at room temperature for 24 hours.

Sodium nucleate prepared from yeast nucleic acid obtained from Merck and Company. Analysis: found—P 6.79.

Dipotassium phenyl phosphate prepared by G. E. Hilbert and N. J. Hipp, formerly of this laboratory. Analysis: calculated for  $\text{K}_2\text{C}_6\text{H}_5\text{PO}_4$ —P 12.39; found—P 12.36 after 8.61 per cent loss in weight upon drying in an Abderhalden apparatus at  $100^\circ\text{C}$ .

Potassium diphenyl phosphate prepared by G. E. Hilbert and N. J. Hipp. Analysis: calculated for  $\text{KC}_{12}\text{H}_{10}\text{PO}_4$ —P 10.76; found—P 10.13.

Potassium diphenyl pyrophosphate prepared by G. E. Hilbert and N. J. Hipp. Analysis: calculated for  $\text{K}_2\text{C}_{12}\text{H}_{10}\text{P}_2\text{O}_7$ —P 15.28; found—P 13.22.

Disodium ethyl phosphate prepared from calcium ethyl phosphate obtained from Monsanto Chemical Company. Since this salt was exceedingly hygroscopic, the sirupy reaction product was made up to volume and analyzed for total and inorganic phosphorus. Found—organic P by difference 96.3 per cent.

Calcium diethyl phosphate from Monsanto Chemical Company recrystallized twice from water. Analysis: calculated for  $\text{CaC}_4\text{H}_{10}\text{P}_2\text{O}_8$ —P 17.92; found—P 17.73.

Triethyl phosphate from Commercial Solvents Corporation. One cubic centimeter contained 0.167 gm. P.

Trimethyl phosphate from Commercial Solvents Corporation. One cubic centimeter contained 0.2607 gm. P.

The procedure adopted was as follows: The phosphates in an amount equivalent to 20 mgm. phosphorus were added to 100 gm. soil in 250-cc. centrifuge bottles and mixed thoroughly, and the mixture was wetted with sufficient water to put it in optimum physical condition. The quantities per 100 gm. soil were for Norfolk 8 cc., for Las Vegas 10 cc., for Cecil 25 cc., and for Chester 38 cc. Where aerobic conditions were desired the bottles were stoppered with cotton plugs, weighed, and stored in a dark room at a temperature of  $25$  to  $28^\circ\text{C}$ . The bottles were reweighed every few days and water was added to compensate for the loss due to evaporation. Duplicate samples were incubated for various periods, usually 3, 7, 14, and 21 days. Where anaerobic conditions were used the procedure was the same except that the bottles containing the soils were placed in vacuum desiccators, which were then evacuated and filled with nitrogen gas that had been freed from traces of oxygen by bubbling through alkaline pyrogallol. The evacuation was twice repeated with subsequent refilling with nitrogen gas in order to insure complete removal of oxygen. The soil samples were then incubated in nitrogen gas at atmospheric pressure concurrently with those run under aerobic conditions. After a desiccator was opened, all soil samples therein were removed for analysis.

At the end of the incubation period water was added to the soils in the centrifuge bottles to make a total water content, including that added initially, of 100 cc. for the Norfolk and Las Vegas soils and 150 cc. for the Cecil and Chester soils. The mixture was then shaken intermittently for about a half hour, a portion of the supernatant liquid decanted and centrifuged, and finally filtered. Samples ranging from 1 to 5 cc. were analyzed immediately for inorganic and total phosphorus by the microchemical method given previously (3). The inorganic phosphate was determined directly, whereas the determination of total phosphorus was preceded by a digestion with perchloric acid to convert the organic into inorganic phosphate. The difference between the two analyses represented the organic phosphorus.

In several preliminary experiments partly anaerobic conditions were obtained by adding sufficient water completely to cover the soils in the bottles, followed by tightly closing these bottles with rubber stoppers for the duration of the experiment. Under these waterlogged conditions the rate of decomposition of glycerophosphate was considerably more rapid than under strictly anaerobic conditions, and hence this method was discarded and the incubation carried out in pure nitrogen gas as already described. Some of these results with waterlogged soils are given below in connection with the discussion of sodium glycerophosphate.

## RESULTS

The behavior of ten organic phosphates in the various soils maintained under aerobic and anaerobic conditions is presented in a number of tables and figures. The tables show the percentages of the added phosphate that could and could not be extracted with cold water after various intervals. The portion of the extractable phosphorus that remained in organic form as well as the portion that had been converted into inorganic phosphate is also shown. In the presentation of these data, the results obtained with each individual phosphate will be considered separately.

### *Sodium glycerophosphate*

The results with glycerophosphate given in table 1 and in figure 1 show that this phosphate is converted into a water-unextractable form rather quickly. In the heavy Cecil soil only 8.2 per cent was recovered in the water extract after 24 hours. In the Norfolk and Las Vegas soils<sup>3</sup> of low colloid content the rate of disappearance from solution was less, but even in these soils only 4.7 and 0.1 per cent, respectively, of organic phosphate was extracted after 7 days under aerobic conditions. Under anaerobic conditions the rate of

<sup>3</sup> The rate of fixation was much more rapid in the Las Vegas soil in the present studies than in the earlier (3) experiments. This was probably due in part to the differences in general experimental procedures used, and also to the fact that in the earlier studies the sodium glycerophosphate was acidified to pH 6 prior to addition, whereas in the present studies it was added without treatment.

disappearance was not quite so rapid. Considerable additional phosphate was also present in the extracts in the inorganic form after 7 days in these coarse-textured soils. This phosphate, formed from glycerophosphate, behaves like any other inorganic phosphate and in heavier soil such as the Cecil would not remain soluble. In this study we are primarily interested in the phosphate only as long as it remains in the organic form.

Experiments with Chester loam, not reported in table 1, showed that as much as 90 per cent of the phosphorus added as sodium glycerophosphate was rendered unextractable in as short a time as 4 hours, and 97 per cent in 1 day. When this soil was steam sterilized and treated with the phosphate solution under sterile conditions the amounts of organic phosphate retained were 65

TABLE 1  
*Rate of disappearance of sodium glycerophosphate from soil solutions as evidenced by the phosphorus content of the extracts*

SOIL	TIME	AEROBIC				ANAEROBIC			
		Percentage of added phosphorus							
		Extracted			Not ex- tracted	Extracted			Not ex- tracted
		Inor- ganic	Or- ganic	Total		Inor- ganic	Or- ganic	Total	
	<i>days</i>								
Norfolk.....	3	36.1	32.1	68.2	31.8	5.2	66.9	72.1	27.9
	7	53.0	4.7	57.7	42.3	5.1	54.7	59.8	40.2
	14	47.1	2.0	49.1	50.9	8.6	30.8	39.4	60.6
	21	44.7	1.7	46.4	53.6	12.9	21.0	33.9	66.1
Las Vegas.....	3	16.9	10.8	27.7	72.3	8.4	35.6	44.0	56.0
	7	16.5	0.1	16.6	83.4	9.6	4.6	14.2	85.8
	14	14.7	0.0	14.7	85.3	4.8	0.6	5.4	94.6
	21	12.2	0.8	13.0	87.0	3.5	0.1	3.6	96.4
Cecil.....	1	0.0	8.2	8.2	91.8				
	3	0.0	0.0	0.0	100.0				

and 78 per cent for the 4- and 24-hour periods, respectively. Steam sterilization, besides killing the microorganisms, undoubtedly also altered the chemical and perhaps the physical properties of the soil to some extent. Nevertheless, the results indicate that soil microorganisms played a minor role in the fixation in this soil.

The rate of disappearance of glycerophosphate from a waterlogged Norfolk soil in comparison with that from a well-aerated soil and also from a soil maintained in pure nitrogen gas is shown in figure 2. As might be expected, the behavior in the saturated soil was intermediate between that in aerobic and strictly anaerobic conditions where the moisture content was optimum. Under anaerobic conditions the organic phosphate extractable after 3 weeks

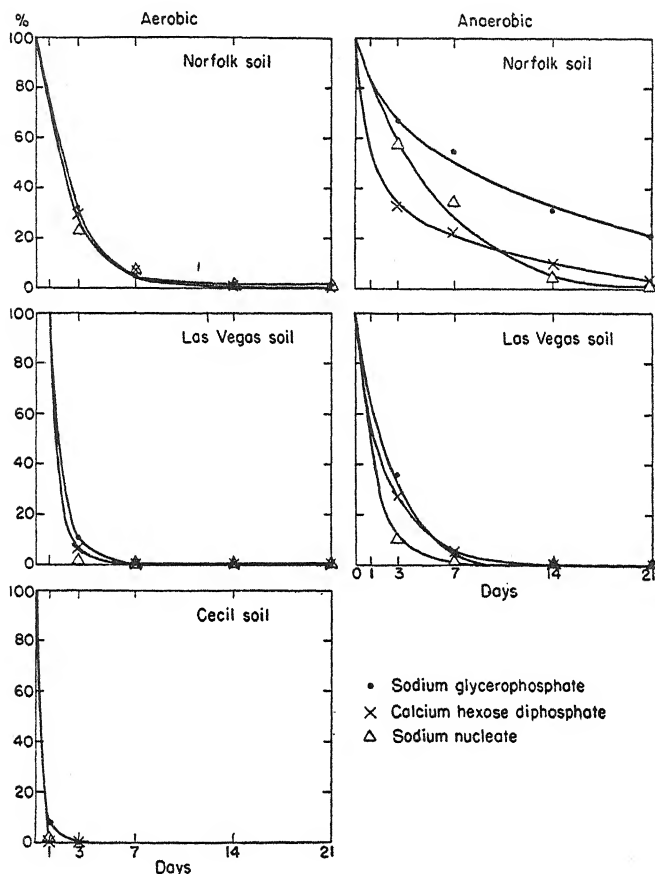


FIG. 1. SOLUBLE ORGANIC PHOSPHATES IN SOILS AT VARIOUS PERIODS FOLLOWING THEIR ADDITION

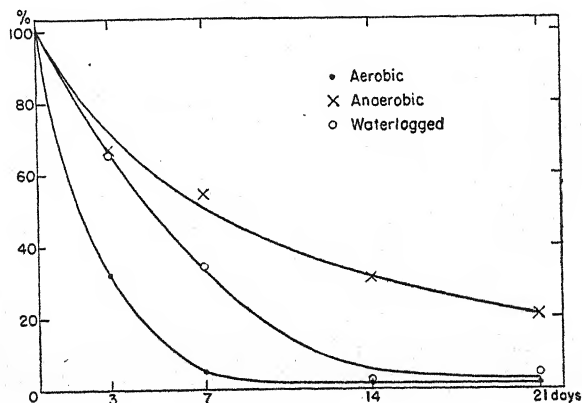


FIG. 2. SOLUBLE ORGANIC PHOSPHATE IN THE NORFOLK SOIL FOLLOWING ADDITIONS OF SODIUM GLYCEROPHOSPHATE

was about the same as in the waterlogged soil after 9 days. Of course the fact that the water content of the waterlogged and the strictly anaerobic soils varied makes it impossible to attribute the observed differences wholly to degree of aeration, but it is probable that the oxygen supply, through its effect on the soil flora, was the predominating factor. Figure 2 would seem to indicate that waterlogged soils are not so completely anaerobic as writers have often asserted or implied, but obviously under field conditions where the soil layer is deeper, oxygen lack would be more in evidence.

TABLE 2

*Rate of disappearance of calcium hexose diphosphate from soil solutions as evidenced by the phosphorus content of the extracts*

SOIL	TIME	AEROBIC				ANAEROBIC			
		Percentage of added phosphorus							
		Extracted			Not extracted	Extracted			Not extracted
		Inorganic	Organic	Total		Inorganic	Organic	Total	
	days								
Norfolk .....	3	12.2	29.7	41.9	58.1	5.7	33.7	39.4	60.6
	7	18.4	4.9	23.3	76.7	4.5	23.1	27.6	72.4
	14	16.8	1.1	17.9	82.1	4.5	9.8	14.3	85.7
	21	15.4	0.3	15.7	84.3	3.2	3.8	7.0	93.0
Las Vegas.....	3	4.7	7.0	11.7	88.3	3.3	27.4	30.7	69.3
	7	5.0	0.4	5.4	94.6	3.4	5.4	8.8	91.2
	14	3.7	0.0	3.7	96.3	2.5	0.7	3.2	96.8
	21	3.3	0.1	3.4	96.6	2.1	0.2	2.3	97.7
Cecil.....	1	0.0	0.2	0.2	99.8				
	3	0.0	0.0	0.0	100.0				

### *Calcium hexose diphosphate*

The behavior of calcium hexose diphosphate in the Norfolk and Las Vegas soils under aerobic and anaerobic conditions is shown in table 2 and in figure 1. The data are in close agreement with those for sodium glycerophosphate; both phosphates are excellent sources of energy for microorganisms, and hence the phosphorus is quickly converted into the inorganic form. Where calcium hexose diphosphate was added to the Cecil soil the change of the phosphate to a fixed form was almost complete within 24 hours.

### *Sodium nucleate*

Table 3 and figure 1 give the results with sodium nucleate, another organic phosphate that is an excellent food for microorganisms. In this case the organic phosphate usually disappeared even more rapidly than did glycerophosphate and calcium hexose diphosphate. This was especially true in the

alkaline Las Vegas soil. In the Cecil soil only 1 per cent was extractable after 24 hours.

TABLE 3

*Rate of disappearance of sodium nucleate from soil solutions as evidenced by the phosphorus content of the extracts*

SOIL	TIME	AEROBIC				ANAEROBIC			
		Percentage of added phosphorus							
		Extracted			Not ex- tracted	Extracted			Not ex- tracted
		Inor- ganic	Or- ganic	Total		Inor- ganic	Or- ganic	Total	
	<i>days</i>								
Norfolk.....	3	47.5	22.4	69.9	30.1	9.2	57.6	66.8	33.2
	7	56.8	7.1	63.9	36.1	12.6	34.6	47.2	52.8
	14	60.9	2.3	63.2	36.8	24.9	4.8	29.7	70.3
	21	56.5	1.8	58.3	41.7	19.4	1.6	21.0	79.0
Las Vegas.....	3	10.9	2.1	13.0	87.0	5.6	10.5	16.1	83.9
	7	11.1	0.2	11.3	88.7	4.3	1.3	5.6	94.4
	14	5.9	0.0	5.9	94.1	3.1	0.3	3.4	96.6
	21	2.3	0.1	2.4	97.6	2.5	0.3	2.8	97.2
Cecil.....	1	0.0	1.0	1.0	99.0				

TABLE 4

*Rate of disappearance of dipotassium phenyl phosphate from soil solutions as evidenced by the phosphorus content of the extracts*

SOIL	TIME	AEROBIC				ANAEROBIC			
		Percentage of added phosphorus							
		Extracted			Not ex- tracted	Extracted			Not ex- tracted
		Inor- ganic	Or- ganic	Total		Inor- ganic	Or- ganic	Total	
	<i>days</i>								
Norfolk.....	3	21.1	49.8	70.9	29.1	19.8	50.7	70.5	29.5
	7	33.3	22.0	55.3	44.7	34.1	22.6	56.7	43.3
	14	31.1	14.0	45.1	54.9	30.0	16.9	46.9	53.1
	21	30.1	12.2	42.3	57.7	30.1	11.4	41.5	58.5
Las Vegas.....	3	6.8	30.2	37.0	63.0	6.0	28.1	34.1	65.9
	7	9.1	9.1	18.2	81.8	7.9	12.0	19.9	80.1
	14	8.0	0.1	8.1	91.9	7.0	0.4	7.4	92.6
	21	7.3	0.2	7.5	92.5	6.4	0.2	6.6	93.4
Cecil.....	1	0.0	14.4	14.4	85.6				
	3	0.0	0.0	0.0	100.0				

#### *Dipotassium phenyl phosphate*

Studies with dipotassium phenyl phosphate are reported in table 4 and in figure 3. It will be noted from the graphs that in both the Norfolk and Las



Vegas soils this phosphate behaved very much the same as the three phosphates just discussed except that it did not disappear from solution quite so rapidly. The rate of conversion to the insoluble form was almost as rapid under anaerobic as under aerobic conditions. This is probably due to the toxicity of phenyl compounds which would tend to decrease the growth of both aerobic

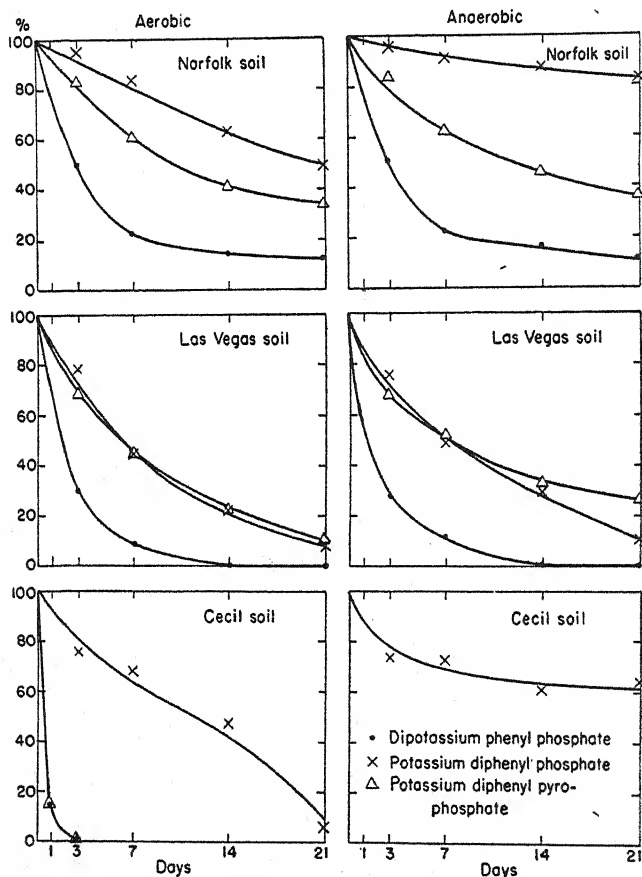


FIG. 3. SOLUBLE PHENYL PHOSPHATES IN SOILS AT VARIOUS PERIODS FOLLOWING THEIR ADDITION

and anaerobic organisms. In the alkaline Las Vegas soil 92 per cent of the phosphorus added as phenyl phosphate could not be extracted with water after 14 days; in the Cecil soil 85.6 per cent was not extracted after 1 day.

#### *Potassium diphenyl phosphate*

The extent to which potassium diphenyl phosphate remains extractable in the three soils is shown in table 5 and in figure 3. This phosphate behaves

very differently from glycerophosphate, hexose diphosphate, and sodium nucleate in that it does not rapidly change into the water-insoluble form. The tendency to remain in solution unchanged was also much greater than was the case with dipotassium phenyl phosphate. Even in the high-colloid Cecil soil about 50 per cent of the diphenyl phosphate was recovered after 2 weeks. The rate of change to a nonextractable form was most rapid in the Las Vegas soil, suggesting that the reaction of a soil is a greater factor than the colloid content in the disappearance of this phosphate from solution.

A number of contributing factors are responsible for the relatively sluggish rate of transformation of the potassium diphenyl phosphate into an inorganic

TABLE 5

*Rate of disappearance of potassium diphenyl phosphate from soil solutions as evidenced by the phosphorus content of the extracts*

SOIL	TIME	AEROBIC				ANAEROBIC			
		Percentage of added phosphorus							
		Extracted			Not ex- tracted	Extracted			Not ex- tracted
		Inor- ganic	Or- ganic	Total		Inor- ganic	Or- ganic	Total	
	days								
Norfolk.....	3	0.2	95.0	95.2	4.8	trace	95.4	95.4	4.6
	7	1.5	83.6	85.1	14.9	0.2	91.2	91.4	8.6
	14	7.0	63.0	70.0	30.0	0.6	87.7	88.3	11.7
	21	11.4	49.4	60.8	39.2	1.1	83.1	84.2	15.8
Las Vegas.....	3	1.7	78.5	80.2	19.8	2.1	75.8	77.9	22.1
	7	5.1	45.1	50.2	49.8	4.2	49.0	53.2	46.8
	14	6.8	22.6	29.4	70.6	6.0	29.6	35.6	64.4
	21	8.0	7.5	15.5	84.5	7.2	10.7	17.9	82.1
Cecil.....	3	0.0	75.2	75.2	24.8	0.0	74.2	74.2	25.8
	7	0.0	68.7	68.7	31.3	0.0	73.1	73.1	26.9
	14	0.0	47.5	47.5	52.5	0.0	61.3	61.3	38.7
	21	0.0	6.1	6.1	93.9	0.0	64.5	64.5	35.5

phosphate. Since this compound is a disubstituted organic phosphate, a twofold hydrolysis would be required to change it completely. The initial hydrolysis appears to be much slower than that encountered with any of the other compounds thus far described, and its hydrolysis product, phenol, would be toxic to soil microorganisms, thereby retarding the final hydrolysis to form the inorganic phosphate.

#### *Dipotassium diphenyl pyrophosphate*

Table 6 and figure 3 show that potassium diphenyl pyrophosphate remains in solution in the three soils for a longer time than does the dipotassium phenyl phosphate but not so long as the potassium diphenyl phosphate in two out of

TABLE 6

*Rate of disappearance of potassium diphenyl pyrophosphate from soil solutions as evidenced by the phosphorus content of the extracts*

SOIL	TIME	AEROBIC				ANAEROBIC			
		Percentage of added phosphorus							
		Extracted			Not extracted	Extracted			Not extracted
		Inor-ganic	Organic	Total		Inor-ganic	Organic	Total	
	<i>days</i>								
Norfolk.....	3	3.5	83.1	86.6	13.4	4.2	83.7	87.9	12.1
	7	9.1	60.8	69.9	30.1	10.2	62.4	72.6	27.4
	14	13.8	41.5	55.3	44.7	11.9	46.1	58.0	42.0
	21	16.1	34.4	50.5	49.5	15.5	36.1	51.6	48.4
Las Vegas.....	3	3.2	69.0	72.2	27.8	3.1	68.1	71.2	28.8
	7	5.3	46.3	51.6	48.4	4.4	52.2	56.6	43.4
	14	7.1	22.9	30.0	70.0	6.1	33.5	39.6	60.4
	21	7.8	11.0	18.8	81.2	5.6	27.0	32.6	67.4
Cecil.....	1	0.0	15.2	15.2	84.8				

TABLE 7

*Rate of disappearance of disodium ethyl phosphate from soil solutions as evidenced by the phosphorus content of the extracts*

SOIL	TIME	AEROBIC				ANAEROBIC			
		Percentage of added phosphorus							
		Extracted			Not ex- tracted	Extracted			Not ex- tracted
		Inor- ganic	Organic	Total		Inor- ganic	Organic	Total	
	<i>days</i>								
Norfolk.....	3	27.2	40.9	68.1	31.9	12.1	55.5	67.6	32.4
	7	53.0	0.0	53.0	47.0	8.4	47.8	56.2	43.8
	14	42.2	0.8	43.0	57.0	7.7	35.9	43.6	56.4
	21	40.6	1.2	42.2	57.8	7.5	29.9	37.4	62.6
Las Vegas.....	3	9.2	37.3	46.5	53.5	5.9	47.5	53.4	46.6
	7	16.8	5.2	22.0	78.0	6.5	27.3	33.8	66.2
	14	14.7	0.6	15.3	84.7	5.4	4.5	9.9	90.1
	21	12.8	0.3	13.1	86.9	4.2	1.3	5.5	94.5
Cecil.....	3	0.0	2.8	2.8	97.2	0.0	4.8	4.8	95.2
	7	0.0	2.5	2.5	97.5	0.0	4.0	4.0	96.0
	14	0.0	0.8	0.8	99.2	0.0	2.0	2.0	98.0
	21	0.0	0.5	0.5	99.5	0.0	1.0	1.0	99.0

the three soils. The pyrophosphate, like potassium diphenyl phosphate, also requires a twofold hydrolysis for its conversion into an inorganic phosphate. The initial hydrolysis, however, involves the conversion of the pyrophosphate

into an orthophosphate, and only in the final hydrolysis is the toxic phenol formed. Chemically, diphenyl pyrophosphate is very stable; it remains unchanged after 7 minutes' heating at 100°C. in normal hydrochloric acid solution (5). Its behavior in soil solution was very different. The rate of its disappearance was most rapid in the Cecil soil, less rapid in the alkaline Las Vegas soil,

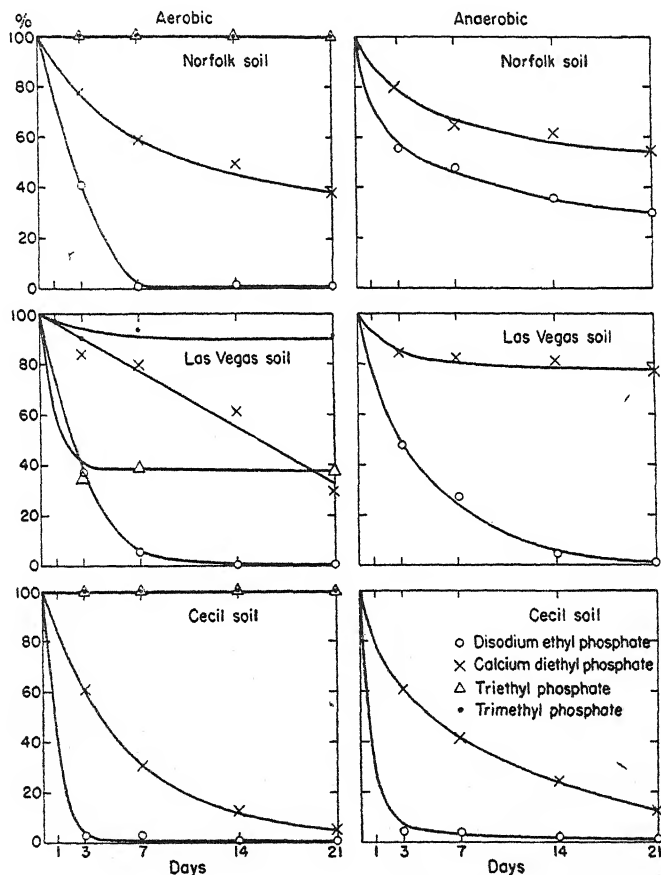


FIG. 4. SOLUBLE ALKYL PHOSPHATES IN SOILS AT VARIOUS PERIODS FOLLOWING THEIR ADDITION

and least rapid in the Norfolk soil. There was no great difference between the results obtained under aerobic and under anaerobic conditions.

#### *Disodium ethyl phosphate*

Where phosphorus was supplied in the form of disodium ethyl phosphate the organic phosphate was transformed into the inorganic form nearly as rapidly as the phosphorus in glycerophosphate or in hexose phosphate. The data, given in table 7 and in figure 4, show that even in the light-textured

Norfolk soil only 41 per cent of the added phosphorus remained in an extractable organic form after 3 days under aerobic conditions and only a trace remained after 7 days. In the Cecil soil very little phosphorus could be extracted after 3 days. Under anaerobic conditions disodium ethyl phosphate disappeared less rapidly, probably because of the decreased activity of microorganisms.

### *Calcium diethyl phosphate*

The data in table 8, which are in part plotted in figure 4, show that diethyl phosphate remains in solution in the three soils for a much longer time than

TABLE 8

*Rate of disappearance of calcium diethyl phosphate from soil solutions as evidenced by the phosphorus content of the extracts*

SOIL	TIME	AEROBIC				ANAEROBIC			
		Percentage of added phosphorus							
		Extracted			Not ex- tracted	Extracted			Not ex- tracted
		Inor- ganic	Or- ganic	Total		Inor- ganic	Or- ganic	Total	
	<i>days</i>								
Norfolk.....	3	2.9	78.0	80.9	19.1	2.3	80.5	82.8	17.2
	7	6.8	58.6	65.4	34.6	5.2	64.3	69.5	30.5
	14	8.8	49.1	57.9	42.1	4.3	61.2	65.5	34.5
	21	9.3	37.5	46.8	53.2	4.1	54.4	58.5	41.5
Las Vegas.....	3	0.8	83.5	84.3	15.7	0.7	84.2	84.9	15.1
	7	0.9	79.6	80.5	19.5	0.6	82.8	83.4	16.6
	14	2.8	61.0	63.8	36.2	0.5	81.3	81.8	18.2
	21	5.0	29.1	34.1	65.9	0.4	76.2	76.6	23.4
Cecil.....	3	0.0	61.2	61.2	38.8	0.0	60.5	60.5	39.5
	7	0.0	30.4	30.4	69.6	0.0	41.3	41.3	58.7
	14	0.0	12.7	12.7	87.3	0.0	24.8	24.8	75.2
	21	0.0	4.8	4.8	95.2	0.0	12.5	12.5	87.5

does glycerophosphate, hexose diphosphate, or sodium nucleate. The rate of disappearance under aerobic conditions was slightly greater than under anaerobic conditions, indicating that microorganisms did attack the material, but very slowly. So far as retention is concerned, the diethyl phosphate behaved in some respects like the diphenyl phosphate and the diphenyl pyrophosphate but with variations for the different soils.

### *Triethyl phosphate*

Triethyl phosphate is a liquid and hence is not physically suitable for ordinary fertilizer use. It also fails to ionize in water solution and probably does not act as a source of phosphorus for plants. Nevertheless it is of scien-

tific interest to know its behavior in soils. Figure 4 gives this information for the three soils maintained under aerobic conditions. It will be noted that in the Norfolk and Cecil soils the material could be recovered completely by water extraction after 21 days; in the Las Vegas soil only 38 per cent remained water-extractable. The retention by the Las Vegas soil may be attributed to a purely chemical reaction involving hydrolysis in the presence of the soil bases. The failure of the unionized triethyl phosphate to be adsorbed by the colloidal Cecil soil is in harmony with the idea that colloidal adsorption involves an ionic reaction. The complete recovery of triethyl phosphate also shows that this material is not utilized by soil microorganisms.

#### *Trimethyl phosphate*

Figure 4 shows that trimethyl phosphate acts like triethyl phosphate, as would be expected since it is also unionized. It was recovered completely from the Norfolk and Cecil soils and to the extent of 87 per cent after 3 weeks from the Las Vegas soil.

#### DISCUSSION

One of the most striking facts brought out by the data presented here is that the common water-soluble organic phosphates, such as sodium glycerophosphate, calcium hexose diphosphate, and sodium nucleate, when added to a soil containing a considerable percentage of colloids, become nonextractable with water nearly as quickly and completely as do soluble inorganic phosphates. In the Cecil soil 92 per cent or more of the phosphorus added in these compounds could not be extracted with water after 24 hours and none after 3 days. Although these phosphates are excellent foods for microorganisms, their retention occurred so soon after addition to this soil that the soil organisms undoubtedly played a secondary initial role. The retention was probably due to colloids. In the lighter Norfolk and Las Vegas soils the rate of disappearance from solution was also rather rapid but less so than in the Cecil. In these lighter soils microorganisms probably hydrolyzed the phosphates to inorganic compounds, which then behaved like ordinary soluble inorganic phosphate fertilizers.

The phenyl phosphates behaved somewhat differently in some cases from the three phosphates discussed in the preceding paragraph. These phenyl compounds are undoubtedly toxic to soil microorganisms, and hence the extent to which they would be retained in a given soil would depend to a great extent on physical and chemical factors. It will be noted from figure 3 that dipotassium phenyl phosphate disappeared from the Cecil soil solution, as evidenced by the water extracts, almost as quickly as did the glycerophosphate, and in the Norfolk and Las Vegas soils less than 25 per cent of the organic phosphorus remained extractable after 1 week. Potassium diphenyl pyrophosphate acted similarly except that the change was not so rapid. Potassium diphenyl phosphate acted like the diphenyl pyrophosphate in the alkaline

Las Vegas soil but tended to stay in solution for a considerably longer period in the two humid soils, especially in the Cecil where 50 to 60 per cent could be extracted after 2 weeks. From these results alone it would seem that this compound might offer advantages over common inorganic phosphates as far as penetrability is concerned. It is very probable, however, that the phenyl phosphates would be toxic to most crops if used in appreciable concentrations.

Calcium diethyl phosphate is another compound that these results would indicate might possibly have some advantages over the inorganic phosphates with regard to movement through a soil. In the Las Vegas soil it remained in solution longer than did potassium diphenyl phosphate, whereas in the Norfolk and Cecil soils the reverse was true. Since this organic phosphate is an ester of ethyl alcohol and phosphoric acid, and since ethyl alcohol is a good food for many microorganisms, it is surprising that it was not converted into water-insoluble phosphate much more rapidly than was found to be the case. It seems unlikely that a compound of this type would be toxic to higher plants in any concentration likely to be used in common fertilizer practice. Field tests with this compound would be of considerable scientific interest. Disodium ethyl phosphate behaved much more like the sugar phosphate and glycerophosphate than like the diethyl phosphate.

The unionized triethyl and trimethyl phosphates, which could be completely recovered from the Norfolk and Cecil soils after 3 weeks, are probably not readily available sources of phosphorus for plants. The basic soils may be an exception as far as hydrolysis is concerned, as the results for the Las Vegas soil indicate. It is pertinent to mention that Conrad (2) observed that triethyl phosphate was toxic to milo.

Other results with organic phosphates, recently reported by Conrad (2), should also be mentioned. He observed that a loamy fine sand (Yolo) retained the phosphorus of phytin but only a small portion of that from glycerophosphate, whereas Aiken loam retained most of the phytin and glycerophosphate as well as sodium nucleate. Triethyl phosphate was retained to some extent by both soils. In earlier studies Spencer and Stewart (4), using Las Vegas loam, found that organic phosphates of the type formula  $R(OH)_x(PO_4M_y)_z$  have greater soil-penetrating power than the common inorganic phosphates.

The experiments reported in the present study merely show to what extent a variety of types of organic phosphates are able to remain in solution in the presence of soils. The availability or toxicity to plants of many of these compounds is not well known. Furthermore, the feasibility of using them in fertilizer mixtures would require a considerable study. It is also very doubtful if any of these or similar materials could be manufactured at sufficiently low cost to make their use practical, assuming that a compound such as calcium diethyl phosphate should prove to have marked advantages over inorganic fertilizers with respect to penetrability.

## SUMMARY

The rates of change to the water-insoluble or at least nonextractable condition of the phosphorus in ten soluble organic phosphates were determined under aerobic and anaerobic conditions in three, and in some cases, four soil types.

In general, the phosphorus in sodium glycerophosphate, calcium hexose diphosphate, sodium nucleate, dipotassium phenyl phosphate, and disodium ethyl phosphate was retained rapidly by Cecil clay loam and usually by Las Vegas loam, whereas retention by Norfolk sandy loam was not so rapid or so complete. The phosphorus in potassium diphenyl phosphate and in potassium diphenyl pyrophosphate usually remained water-soluble for a considerably longer period. The unionized triethyl and trimethyl phosphates could be completely recovered from two of the three soils after 3 weeks. Phosphorus supplied as calcium diethyl phosphate tended to remain in the water-soluble form for several days, and might possibly have some advantages over inorganic phosphates from the standpoint of penetrability.

These studies showed that some of the organic phosphates, such as glycerophosphate, were retained by heavy soils so tenaciously and so quickly after addition that colloidal action rather than microorganisms must have played the primary role.

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# THE FOUR-ELECTRODE RESISTANCE METHOD FOR MEASURING SOIL-MOISTURE CONTENT UNDER FIELD CONDITIONS<sup>1</sup>

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Under conditions requiring irrigation, where both water and labor are expensive, it is desirable to have some record of changes in moisture conditions in the soil. If the soil is irrigated while it is still high above the permanent wilting percentage, water probably will be wasted, and if it is not irrigated until some time after the soil in the root zone has reached the permanent wilting percentage, the growth of the plant may be undesirably checked. The standard procedure of determining the moisture content at given points of the soil in the field requires taking soil samples from the field into the laboratory, then weighing, drying, and again weighing them before the moisture content can be calculated. When taken in sufficient quantities and with sufficient care, this seems to be the most reliable method used to date. When carried out in sufficient detail, however, it is laborious. The development of a reliable method for measuring moisture content without having to remove the soil sample is, therefore, highly desirable. One of a number of methods being investigated by the authors for measuring the moisture content of a soil in place is that depending on the relation between the electrical resistance of a soil and its moisture content.

Whitney, Gardner, and Briggs (1, 2, 8) carried out rather elaborate investigations on the electrical resistance of soils. Their work, for the major part, was done under humid conditions. Their method was to use only two electrodes in the soil and an alternating current to avoid polarization. They reported the carbon electrodes as most satisfactory. The general conclusion drawn by soil workers from the above results was that the variations in salt content were too great to allow the use of the resistance method for measuring variations in moisture content, although the original authors seem not to have drawn this specific conclusion.

Another objection to the two-electrode method of measuring the electrical resistance of soil is that the contact resistance between the electrodes and the soil may be erratic, for any expansion or contraction of the soil around the electrodes will lower or raise that contact resistance. The two-electrode method measures the sum of both the soil resistance and the contact resistance

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between the electrode and the soil. The latter is very erratic and unreproducible and its elimination is highly desirable.

The standard method of eliminating the contact resistance between the electrodes and the material is to use four electrodes. This appears not to have been recognized by the earlier workers in soils. McCorkle (6), however, recognized the great source of error inherent in the two-electrode method when used in measuring the resistance of soils, and hence he made a brief investigation using the four- or multiple-electrode method. He reported results of his work on one soil but failed to identify the soil sufficiently well to enable the reader to interpret the results in terms of the range of soil moisture available to plants. His chief contribution lay in pointing out that the four-electrode method has certain advantages in soil studies. He, like the earlier investigators (5, 7), used carbon electrodes.

Goldsmith (3) also used four electrodes but not in such a way as to eliminate the erratic contact resistance between the electrode and the soil.

The purpose of the present investigations was to determine under field conditions the feasibility of using the electrical resistance of soils, determined with four electrodes, as an indicator of the moisture content of the soil. In the following pages the term *soil-moisture cycle* signifies the continuously varying state beginning with a complete irrigation of the soil, then passing through the drying stage to the permanent wilting percentage, due to the extraction of water by plants, and ending with a complete irrigation.

The most obvious source of difficulty with any electrical resistance method is the variation of electrical resistance with the variation in amount of dissolved material in the soil solution, the effect of the variation of soil temperature being negligible below the first foot in the soil during the growing season. A comparison of the electrical resistance, at a given moisture content, in different *cycles*, furnishes the type of data required for answering the question as to whether the variations in salt content are too great to permit the use of the four-electrode resistance method as an indicator of soil-moisture content. Another question for which an answer was desired was the nature of the variation in the electrical resistance of the soil, as measured by the four-electrode method, in the neighborhood of the permanent wilting percentage.

#### THEORY

We shall derive the expression to be used in determining the electrical resistance of the soil by the four-electrode method (6). Consider four electrodes (fig. 1), equally spaced at a distance  $\rho$  apart and all lying in the same plane at a given level. Let  $R_1 \dots R_4$  represent the electrical resistances in ohms as measured across the terminals of the electrodes shown in the figure. Each of these resistances may be considered as having three component resistances: a contact resistance in going across the boundary from the electrode into the soil, the resistance of the soil itself, and another contact resistance in going from the soil to the other electrode. Let  $r_1, r_2, r_3$ , and  $r_4$  represent the contact

resistance at each of the electrodes, respectively, and  $\mu_1 \dots \mu_6$  the resistances of the soil itself, corresponding to the measured resistances  $R_1 \dots R_6$ . We then have the six following equations:

$$R_1 = r_1 + \mu_1 + r_2$$

$$R_2 = r_1 + \mu_2 + r_3$$

$$R_3 = r_1 + \mu_3 + r_4$$

$$R_4 = r_2 + \mu_4 + r_3$$

$$R_5 = r_2 + \mu_5 + r_4$$

$$R_6 = r_3 + \mu_6 + r_4$$

Subtracting the sum of the second and fifth equation from the sum of the first and sixth we get:

$$(R_2 + R_5) - (R_1 + R_6) = (\mu_2 + \mu_5) - (\mu_1 + \mu_6)$$

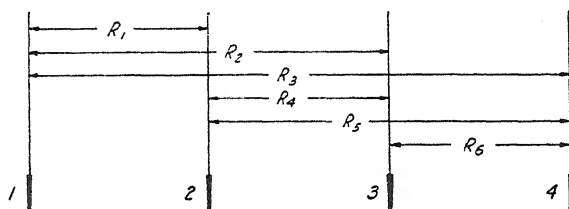


FIG. 1. ARRANGEMENT OF ELECTRODES ILLUSTRATING THE MEANING OF VARIOUS RESISTANCES USED IN THE DISCUSSION

Since we are considering a uniform medium and since the electrodes are uniformly spaced, we have  $\mu_2 = \mu_5$  and  $\mu_1 = \mu_6$ . Hence

$$(R_2 + R_5) - (R_1 + R_6) = 2(\mu_2 - \mu_1) \quad (A)$$

To solve this equation, we must express  $\mu_2$ ,  $\mu_5$ , and  $\mu_6$  in terms of  $\mu_1$ . To do this, we shall consider two extreme cases which are more amenable to mathematical treatment and between which our actual case lies. The same conclusion will be found to follow from both of the two extreme cases; the same must therefore apply to the actual case.

Consider first the formula for the resistance  $\mu$  between two spherical electrodes (4, pp. 350-352), radii  $a$  and  $b$ , at a great distance  $\rho$  apart, in an infinite conducting medium. We have

$$\mu = \frac{\tau}{4\pi} \left\{ \frac{1}{a} + \frac{1}{b} - \frac{2}{\rho} \right\}$$

where  $\tau$  is the specific resistance of the medium. Hence

$$\mu_2 = \mu_1 \frac{\left[ \frac{1}{a} + \frac{1}{b} - \frac{1}{\rho} \right]}{\left[ \frac{1}{a} + \frac{1}{b} - \frac{2}{\rho} \right]} = k_1 \mu_1 \quad (B)$$

Thus the resistance  $\mu_2$  is at all times proportional to  $\mu_1$  as long as the foregoing conditions are satisfied.

Let us now consider the formula for the resistance  $\mu$  per unit length between two straight parallel cylindrical electrodes (4, pp. 350-352), of radii  $a$  and  $b$ , placed with their centers at a great distance  $\rho$  apart, in an infinite conducting medium. We have

$$\mu = \frac{\tau}{2\pi} \ln \frac{\rho^2}{ab}.$$

Hence

$$\mu_2 = \mu_1 \frac{\ln \frac{(2\rho)^2}{ab}}{\ln \frac{\rho^2}{ab}} = k_2 \mu_1 \quad (C)$$

Thus again the resistance  $\mu_2$  is at all times proportional to  $\mu_1$ .

Since our actual case lies between the two hypothetical cases considered above (possibly closer to the first), we may write for the actual case,

$$\mu_2 = k\mu_1.$$

Equation (A) then becomes

$$\begin{aligned} (R_2 + R_5) - (R_1 + R_6) &= 2\mu_1 (k - 1) \\ \mu_1 &= \frac{(R_2 + R_5) - (R_1 + R_6)}{2(k - 1)} = K[(R_2 + R_5) - (R_1 + R_6)] \quad (D) \end{aligned}$$

where  $K$  depends only on the distance apart and shape of the electrodes, and where the erratic behavior of the contact resistances  $r_1 \dots r_4$  between the electrode and soil has been entirely eliminated.

Formulas (B) and (C) were derived for an infinite medium, whereas we are applying it to a case where the departure from infinity of the medium becomes greater as the surface of the soil is approached. The constant  $K$  in equation (D), therefore, depends more on the depth of the electrodes as these are placed closer to the soil surface. At the lower depths, equation (D) should be independent of depth if the medium is homogeneous. For the present purpose we are not interested in evaluating  $K$  in equation (D), although this can be done if desired.

From the nature of  $K$ , it is immaterial whether we use  $\mu_1$  or the numerator on the right-hand side of equation (D) as a measure of the electrical resistance of the soil. In the future, therefore, when we speak of the electrical resistance of the soil by the four-electrode method, we shall, for convenience, be dealing only with

$$(R_2 + R_5) - (R_1 + R_6).$$

#### PRESENT PROCEDURE

For large-scale work, it is desirable to have some convenient means of installing the electrodes without an excessive amount of work. For this reason,

a soil tube was used to drill the holes for the electrodes, and the electrodes were designed to be placed in the soil-tube holes. The electrodes were inserted in the holes after these had been partly filled with a thin mud made from their original soil contents. As the excess water surrounding the electrode seeped away, the mud settled around the electrode. After considerable settling had taken place, the electrode was pushed a bit farther into the wet soil so as to make better electrical contact. The rest of the hole was then filled with earth.

The necessity for using some force in pressing the electrodes even a short distance into the soil proved to be a handicap when carbon electrodes were used, for these were so fragile that many of them broke off even when great care was exercised in installing them. The carbon rods used for electrodes were 1 foot in length and  $\frac{1}{2}$  inch in diameter. They were turned on a lathe to vary from  $\frac{1}{4}$  inch at the lower tip to the full  $\frac{1}{2}$  inch at the upper end. A small shaft was turned down on the large upper end so that it could be inserted in the end of a  $\frac{1}{4}$ -inch pipe. A rubber garden hose completely jacketed the pipe, to which it was securely wired, both at the bottom near the carbon electrode and at the top projecting out of the ground. The rubber hose prevented the soil above the carbon electrode from coming into electrical contact with the pipe.

We compared the carbon electrodes with another far more sturdy set of electrodes made by hammering out a 1-inch square iron rod so that it became a wedge 1 inch square at the upper end, tapered to  $\frac{1}{4}$  inch square at the lower end, and 1 foot long. The long end was welded to a  $\frac{1}{4}$ -inch pipe. Thus strong metal electrodes were substituted for the fragile carbons. The metal electrode and part of the  $\frac{1}{4}$ -inch pipe were then tinned by a commercial tinning plant to prevent rusting. The  $\frac{1}{4}$ -inch pipe was covered by rubber hose in the same manner as were the pipes attached to the carbon electrodes. The tinned iron electrodes showed no measurable difference in electrical characteristics from the carbon ones, throughout the season, and hence it is felt that the iron electrodes are much to be preferred since they are less expensive and far more sturdy.

The two plots selected for the test were on Yolo sandy loam having some variation in texture from one depth to another but being fairly uniform at a given depth. To determine the electrical resistance at a given depth in the soil, the four electrodes were all placed at the desired depth in a straight line and 40 inches apart. Electrodes were placed at the four following depths:  $1\frac{1}{2}$ ,  $3\frac{1}{2}$ ,  $5\frac{1}{2}$ , and  $9\frac{1}{2}$  feet.

Any resistance method under field conditions, of course, measures the resistance of a lens of soil, and hence one cannot say that the results observed are the resistances at any specific level, since the electric lines of force probably, to some extent, spread above and below this level. If one is interested in the average moisture conditions, this is an advantage.

The Wheatstone bridge used in making the measurements was built in the laboratory using a constant frequency output of 1,000 cycles per second.

A variable condenser covering a wide range was necessary to compensate for the capacitance of the electrodes in the soil so that a null could be attained in the telephone receiver of the Wheatstone bridge. One stage of amplification was used before the current was passed through the telephone receivers. The equipment, though rather bulky for routine work, was very sensitive, enabling one to determine resistances to four significant figures.

Throughout the entire season, careful and frequent soil-moisture determinations were made, according to standard procedure, at the different depths, paralleling the resistance measurements. These samples were taken with a soil tube and were dried in the oven at 110°C.

#### RESULTS AND DISCUSSION

It was found that the electrical resistance between any two electrodes varied widely and apparently in no very consistent manner with variations in moisture content. Thus if two electrodes only had been used, no very consistent results would have been expected. By the use of the four-electrode method, however, the variations in the contact resistance between the electrodes and the soil were eliminated, and the resistances of the soil between the electrodes were determined with surprising consistency.

The results for the entire season taken from a Sudan grass plot are shown in figure 2. A similar set of curves was obtained from a sugar beet plot in cooperation with the Spreckels Sugar Company. Since the present tests were started in the season of 1940, the data on but one and one-third cycles, as defined earlier, are available. It will be noticed that although resistance determinations are reported for only five different depths, soil moisture determinations are reported for ten different depths at 1-foot intervals. Thus the sloping broken line for the 2-foot section indicates the variation in resistance of the soil with time at an average depth in the soil of  $1\frac{1}{2}$  feet; whereas the sloping solid line for each horizontal foot section indicates the variation in moisture content with time. The broken sloping lines at the 4-foot, 6-foot, and 10-foot sections likewise indicate the variation in electrical resistance, but at average depths of  $3\frac{1}{2}$ ,  $5\frac{1}{2}$ , and  $9\frac{1}{2}$  feet.

The horizontal broken lines labeled "M. E." indicate the average moisture equivalents for each 1-foot section. Previous to the second irrigation, the moisture-content curves become asymptotic to a horizontal in the upper layers. The moisture contents corresponding to these comparatively flat parts of the curves are the permanent wilting percentages. On the other hand, the comparatively flat parts of the moisture-content curves after the second irrigation do not represent the permanent wilting percentages, since there was virtually no transpiration during this period. The heavy vertical line at September 23 indicates the date of the only irrigation given during the season except the thorough irrigation given the plot of ground immediately after all the electrodes had been installed.

Even though the moisture curves in the surface 4 feet tend to flatten during

the fore part of August, the resistance curves continue to climb without any very appreciable change of soil-moisture content. This, of course, means that the small changes in moisture content taking place in the vicinity of the permanent wilting percentage cause comparatively large changes in electrical resistance. This was expected from results published by McCorkle (6).

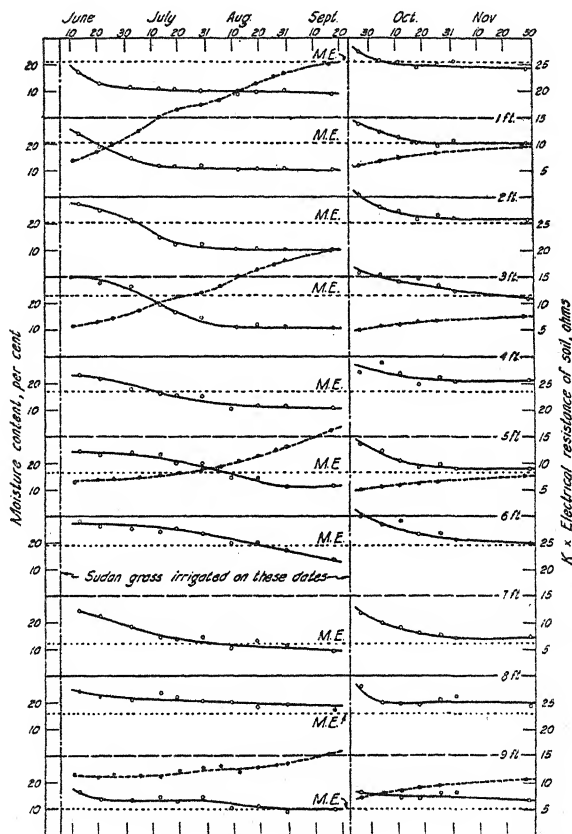


FIG. 2. VARIATION OF ELECTRICAL RESISTANCES AND MOISTURE CONTENTS AS THESE VARY WITH DEPTH AND TIME ON THE SUDAN GRASS PLOT

The curves in figures 3 and 4 indicate the variation in resistance with moisture content as measured at the 2-foot and 4-foot sections. They were obtained from figure 2 by plotting the resistance at different times against the corresponding moisture contents. The points indicated by the open circles represent determinations made after the first irrigation, whereas those represented by solid circles represent the same data after the second irrigation.

The curves or points of figures 3 and 4 corresponding to the second cycle fall reasonably closely on those of the first. That is, when the soil during the



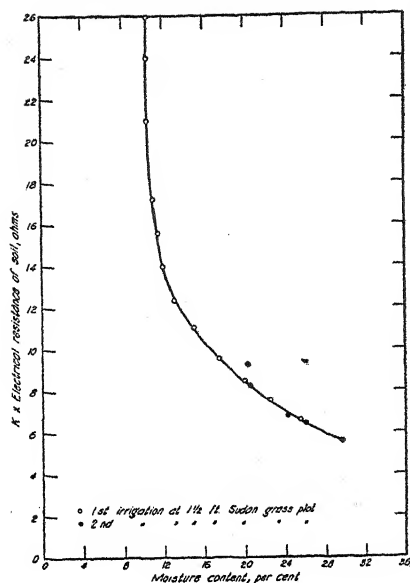


FIG. 3

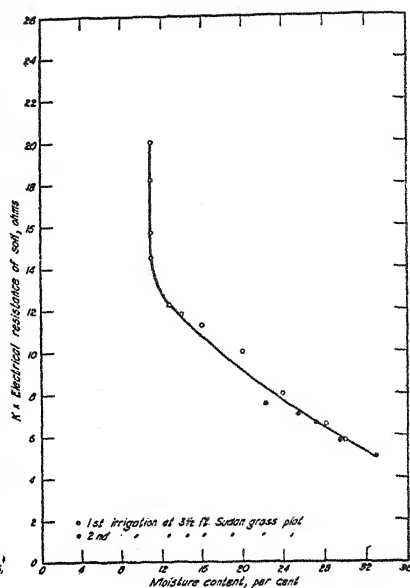


FIG. 4

FIG. 3. VARIATION OF ELECTRICAL RESISTANCES OF SOIL WITH MOISTURE CONTENT IN THE SECOND-FOOT SECTION ON THE SUDAN GRASS PLOT

FIG. 4. VARIATION OF ELECTRICAL RESISTANCES OF SOIL WITH MOISTURE CONTENT IN THE FOURTH-FOOT SECTION ON THE SUDAN GRASS PLOT

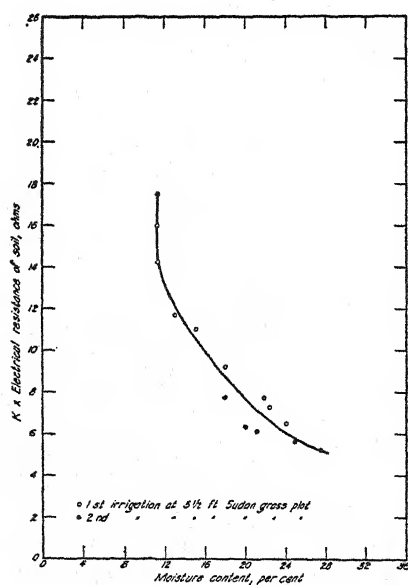


FIG. 5

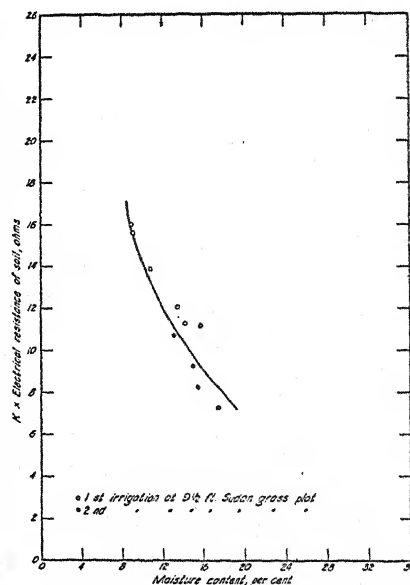


FIG. 6

FIG. 5. VARIATION OF ELECTRICAL RESISTANCES OF SOIL WITH MOISTURE CONTENT IN THE SIXTH-FOOT SECTION ON THE SUDAN GRASS PLOT

FIG. 6. VARIATION OF ELECTRICAL RESISTANCES OF SOIL WITH MOISTURE CONTENT IN THE TENTH-FOOT SECTION ON THE SUDAN GRASS PLOT

second cycle takes on the moisture contents it had during the first, it also takes on approximately the same electrical resistance. This would seem to indicate that the electrode method has promise as a way of measuring or indicating the soil-moisture content. We also conclude from the close coincidence of the two sets of points that the variation in resistances due to variation in salt content or any other variables except moisture, under the conditions in the experiment, are not highly important at least in the top 4 feet of soil. Figures 5 and 6 corresponding to the sixth and tenth foot do not show such close agreement between the two sets of points. Whether this is due to irregularities in the moisture-content determinations or to some other factors such as the downward leaching of salts cannot, at present, be stated.

Before definite conclusions can be drawn as to the practicability of this method, it must be tried under a wide range of field conditions. This work is now in progress and will be continued through next summer.

#### SUMMARY

The four-electrode method was used in measuring the electrical resistance of the soil as a function of moisture content in both a Sudan grass and a sugar beet plot.

Tinned iron electrodes were found to be more sturdy and convenient than carbon and proved to be entirely satisfactory.

There was no sudden increase in the electrical resistance of the soil as the plant continued to remove water in the vicinity of the permanent wilting percentage.

When the electrical resistances of a soil supporting a crop are plotted as a function of moisture content, a curve is obtained showing a rapid increase in resistance with decreasing moisture content in the neighborhood of the permanent wilting percentage. This curve appears to be asymptotic to the moisture content corresponding to the permanent wilting percentage.

A comparison of the resistances at the same moisture content before and after irrigation indicates that the variations in resistances due to factors other than moisture, under the conditions of this experiment, are not great in the top 4 feet of soil. This would seem to indicate that the four-electrode method of indicating variations in soil-moisture content has promise.

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# A PRESSURE-MEMBRANE EXTRACTION APPARATUS FOR SOIL SOLUTION<sup>1</sup>

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The composition of the soil solution has been given considerable attention in connection with soil fertility and soil salinity investigations, and a number of different methods have been used for sampling the soil solution. It is the purpose of this paper to review briefly these methods and to present preliminary results on a new experimental procedure which gives promise of being useful for studying physical as well as chemical properties of the soil solution over the plant growth range of moisture contents.

## REVIEW OF EXTRACTION METHODS

Methods for obtaining samples of the soil solution may be briefly classed under the following headings:

1. *Displacement.* The soil solution may be removed and replaced by a fluid which is caused to move into the soil pore spaces. Liquids are commonly used, but the displacement may be produced by a gas if the soil is saturated. The displacement method was reviewed and improved by Parker (18) and has been extensively used (3, 4, 5, 6, 7, 11, 20, 21, 25). Good evidence for the reliability of the method has been given by Burd and Martin (3), but the range of soil textures and moisture contents on which it can be used successfully is definitely limited. Only exceptional soils can be displaced at moisture contents near the wilting point, and in many instances soils which are readily puddled require excessive periods for the displacement process.

2. *Compaction.* Liquids may be removed from a porous medium if the pore space can be sufficiently reduced by compaction. This method is commonly used in commercial filter presses for extracting moisture from plant residues and ceramic clays, and has been used on soils by a number of workers (9, 15, 22). Pressures of thousands of pounds per square inch are required to reduce the moisture content of soils to near the wilting point; because of the effect of high pressures on solubility, the method has been subjected to rather serious criticism by Northrup (16).

<sup>1</sup> Contribution from the U. S. Regional Salinity Laboratory, Bureau of Plant Industry, Riverside, California.

<sup>2</sup> Senior soil physicist. The author is indebted to P. E. Skaling and L. R. Weaver for assistance in the construction and testing of the apparatus.

3. *Centrifugation.* Water may be caused to move through and out of soil by centrifugation, and this procedure is commonly used for drying soil to standard conditions (2, 17, 24). Attempts have been made by a number of workers to obtain samples of soil solution by centrifugation, but a satisfactory procedure has not yet been developed.

4. *Molecular adsorption.* Gardner, Whitney, and Kezer (10) effected the extraction of solutions from slick spot soils by contact with rolls of dry filter paper. The transfer of solution from the soil to the paper was hastened by compaction in a cylinder with movable pistons. Neither compaction nor centrifugation will remove water from moist soil until the pressure in the soil water is increased to atmospheric pressure at the place provided for outflow; that is, the soil must become substantially saturated at this place. The molecular adsorption method is suitable for supplementing compaction or centrifugation methods when soil moisture contents are too low to satisfy the atmospheric pressure outflow condition.

5. *Suction.* The removal of water from soil by suction is a common procedure, accomplished by connecting the liquid phase of water in soil with liquid water at lower pressure. The mechanism for maintaining this pressure difference between extracted water and water in the soil is usually a porous ceramic wall or other membrane which, when wet, is readily permeable to water but not to air. The suction method as suggested by Briggs and McCall (1) makes it possible to extract moisture from soil until the negative pressure in the soil water is about one atmosphere. This pressure limitation arises from the fact that without elaborate precautions it is not possible in ordinary apparatus to reduce the pressure in liquid water below the aqueous vapor pressure. Porous ceramic cups have been used for extracting solutions from soil samples for fertilizer investigations (13) and for sampling submerged soil solutions (12). Adsorption of ions by the ceramic cell or other membrane material must be considered whenever the suction method is used (19).

The pressure limitation in the suction method may be avoided by increasing the gas pressure in the soil air. This has been done in connection with soil-moisture sorption-curve work by S. J. Richards (23) and has also been done by Lauritzen (14) for increasing the exudate from decapitated potted plants.

#### PRESSURE-MEMBRANE EXTRACTION APPARATUS

The apparatus which has been developed at this laboratory for removing solutions from soils makes use of the gas pressure modification of the suction procedure. The soil from which moisture is to be removed is placed in a chamber in which the gas pressure is increased above atmospheric pressure. The side of the chamber which supports the soil consists of a Cellophane membrane supported on a brass screen and a brass plate in such a way that any solution passing through the membrane is conducted away at atmospheric pressure and trapped under oil. In this way the moisture content of the soil in contact with the membrane will be reduced by the amount that would be

necessary under normal atmospheric conditions to make the pressure deficiency of the soil water equal to the excess gas pressure in the extraction chamber.

It is not difficult to see why excess gas pressure applied on top of a layer of saturated soil should cause the soil water to move through an underlying membrane which is subjected only to atmospheric pressure on the side opposite to the soil. It is not entirely obvious why such a membrane should continue to extract moisture from soil after a continuous gas phase has been established throughout the soil mass and the soil-water system touches the membrane only at comparatively isolated points. The fact that the excess gas pressure can be maintained in the chamber indicates that surface tension action inhibits gas leakage through the membrane pores and there is established in the gas-liquid interface at the upper surface of the membrane an equivalent curvature determined by the excess gas pressure. The comparatively free movement of water through the membrane maintains this same curvature in the water interface at the contact points between the membrane and the soil. There is thus set up a curvature gradient across the layer of soil in contact with the membrane. It is the pressure gradient corresponding to this curvature gradient that moves the water or solution through the unsaturated soil toward the membrane. When equilibrium is reached and passage of water through the membrane ceases, the interface curvature of the water throughout the soil will be the same as at the membrane.

The moisture extraction chambers consist essentially of cylindrical sections clamped between flat steel plates. Two sizes were used in the work reported in this paper. In the small chamber the cylinder was  $2\frac{7}{8}$  inches outside diameter with a wall thickness of  $\frac{3}{16}$  inch. The end plates were  $3\frac{1}{2}$  inches square,  $\frac{1}{2}$  inch thick, and were clamped by four  $\frac{3}{8}$ -inch bolts. The cylinder for the large chamber was 12 inches outside diameter with a wall thickness of  $\frac{1}{4}$  inch. The circular end plates were 14 inches in diameter,  $\frac{5}{8}$  inch thick, and were clamped by eight  $\frac{5}{8}$ -inch bolts. Tripod legs were screwed into the lower plate.

Figure 1 shows the constructional details for the large chamber. To prevent displacement of the gaskets by gas pressure, the rectangular groove shown in the sectional view was cut in the face of the cylinder and the annular rubber gaskets were attached to the cylinder with rubber-to-metal bonding cement. The brass screen is held taut on the brass plate by the smooth annular ring of solder which also serves as the bearing surface for the gasket. A short length of 0.040-inch bore copper tubing is riveted and soldered into the center of the brass plate so that the upper end of the copper tube is flush with the upper surface of the brass plate. This tube, passing downward through the brass plate and the lower steel plate, serves as the outlet for fluids which pass through the cellophane membrane from the soil chamber. The three lugs brazed to the cylinder make it possible to screw the cylinder to the lower steel plate and thus hold the Cellophane and gasket in place while the chamber is being loaded with soil.

The tendency for soil to shrink out of contact with the membrane during dehydration may be prevented by inserting a soft rubber diaphragm between the cylinder and the upper plate. A constant compressive force may thus

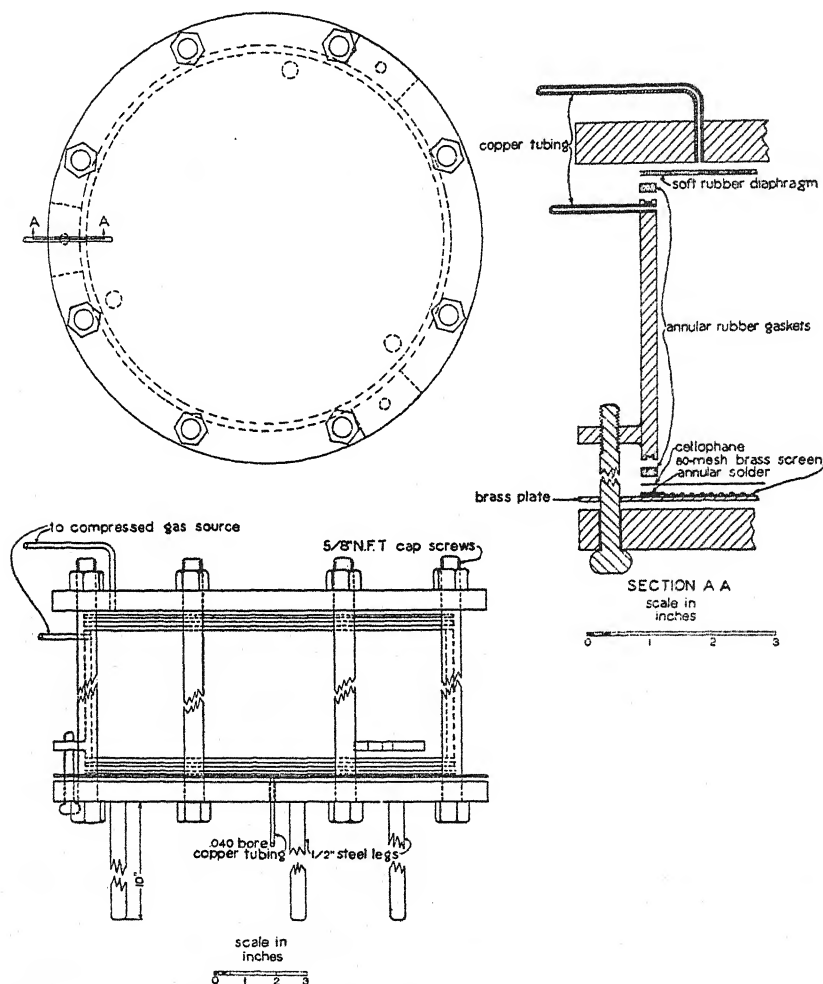


FIG. 1. PRESSURE-MEMBRANE EXTRACTION APPARATUS

be maintained on the soil by keeping the gas pressure above the diaphragm somewhat in excess of the extraction pressure.

The most suitable height for the cylindrical section of the extraction chamber will depend on the nature of the extraction work to be carried on. Cylinder heights of  $\frac{1}{2}$ , 2, and 4 inches have been found convenient for the work in progress at this laboratory.

## MEMBRANE CHARACTERISTICS

Representative data on the permeability of plain transparent Cellophane are shown in table 1. Thickness values for the three gauge numbers commonly available are also given. It may be seen from the data that water transfer through Cellophane is not quite proportional to the pressure difference over the pressure ranges used. The permeability varies somewhat from one sheet to another, and tends to increase slightly during water transfer. The gas permeability measurements were made on the wet membrane immediately following the water-flow tests.

Only occasionally at the beginning of an extraction process have Cellophane membranes developed leaks, and these have been ascribed, for the most part,

TABLE 1  
*Permeability of plain transparent Cellophane to water, air, and nitrogen*  
Expressed as  $\text{cm}^3 \text{ cm}^{-2} \text{ sec}^{-1} \text{ atmos}^{-1} \times 10^6$

GAUGE NUMBER	THICK- NESS	TEMPERA- TURE	DRIVING PRESSURE IN ATMOSPHERES							
			2	4	6	8	2	4	6	8
			Water				Air			
	<i>inches</i>	<i>°C.</i>								
300	.00088	22.0	10.8	9.74	9.33	9.18	7.23	7.10	6.77	6.36
450	.0012	21.5	11.6	10.5	9.95	9.14	5.97	5.60	5.32	5.10
600	.0017	22.5	9.05	8.60	7.80	7.77	5.15	5.00	4.80	4.45
			DRIVING PRESSURE IN ATMOSPHERES							
			3.4	6.8	13.6		3.4	6.8	13.6	
			Water				Nitrogen			
300*	.00088	27.5	17.5	19.6	17.0		150	208	348	
450*	.0012	28.0	14.2	13.8	12.3			4.76	3.92	
600*	.0017	26.2	7.88	7.85	6.80		1.91	3.48	2.82	

\* Cellophane obtained from a different supply house was used in these tests.

to faulty handling. The high permeability to nitrogen shown for the No. 300 sheet in table 1 is exceptional and in this case probably indicates a progressive failure of the membrane. Gas leaks through the membrane are rather likely to develop if the extraction process is continued for more than three or four days.

During preliminary tests it became apparent that solutions from certain saline soils markedly decreased the membrane permeability. It was found, for instance, that the permeability of a sheet of No. 600 Cellophane to 1 *M*  $\text{Na}_2\text{CO}_3$  was exactly half the value obtained for distilled water.

Medium and fine grades of Zsigmondy Ultrafein filters were tested but were not found to be appreciably better than Cellophane and are much more expensive.



## SOLUTION EXTRACTION TESTS ON SOILS

Preliminary tests indicate that the pressure-membrane apparatus may be used for extracting soil solutions over the whole plant growth range of soil moisture contents. Several soils were available for which the permanent wilting percentage had been determined by the sunflower method (8).

The soils were air-dried and had been passed through a 2-mm. sieve. A layer of dry soil approximately 1 cm. deep was spread on No. 600 Cellophane in the large chamber and wetted with distilled water. For these experiments the cylindrical section of the chamber used was  $\frac{1}{2}$  inch high. The soils were allowed to stand from 1 to 2 hours with an excess of water before the chamber was closed and a nitrogen pressure of 16 atmospheres (235 pounds per square inch) applied. This pressure was maintained constant by a regulator valve.

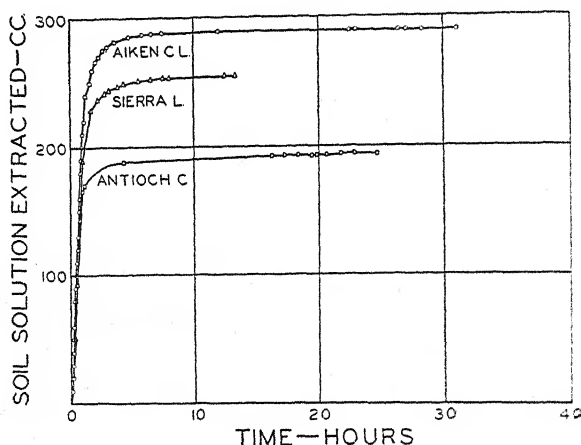


FIG. 2. SUMMATION OF THE SOIL SOLUTION EXTRACTED AS A FUNCTION OF TIME FOR A LAYER OF SATURATED SOIL 29.2 CM. IN DIAMETER WITH AN EXTRACTION PRESSURE OF 16 ATMOSPHERES. THE SOIL DEPTHS WERE: AIKEN, 1.0 CM.; SIERRA, 1.0 CM.; AND ANTIOCH, 0.5 CM.

From the curves in figure 2, it appears that the extraction rate is high and approximately constant until the water table in the soil layer reaches the membrane. The rate of delivery of water to the membrane then depends on flow through the unsaturated soil.

Antioch clay is an extremely impervious soil and shrinks considerably during dehydration. The curve for this soil shown in figure 2 was obtained for a 5-mm. layer on which a soft rubber diaphragm maintained a compressive stress of 10 pounds per square inch after the first half hour of extraction.

It is a matter of considerable interest that for the 5- to 10-mm. layers of soil that have been tried, moisture equilibrium with the 16-atmosphere membrane is attained within 24 to 36 hours, and as indicated in table 2, the equilibrium moisture contents have been below the wilting points for the respective

soils. This seems to supply important new information on the possible rate of the movement of water to plant roots in dry soils.

The curves in figure 3 show extraction rates from 10-cm. layers of soil initially containing moisture at  $\frac{1}{2}$  atmosphere tension. Air pressure on the membrane was 6.8 atmospheres (100 pounds per square inch) and a compressive stress of 20 pounds per square inch was maintained by a pressure of 120 pounds

TABLE 2  
*Moisture content values for three soils under various standard conditions*

SOIL	MOISTURE EQUIVALENT	WILTING COEFFICIENT	MOISTURE AT $\frac{1}{2}$ ATMOS. TENSION	MOISTURE AT 16 ATMOS. TENSION
Sierra loam.....	8.6	3.7	6.0*	3.27*
Aiken clay loam.....	36.2	22.0	29.5	21.24
Antioch clay.....	21.0	12.0	20.6	11.2

\* Although taken from the field at a later date, this sample was from the same depth and location as the sample on which the moisture equivalent and the wilting coefficient were determined.

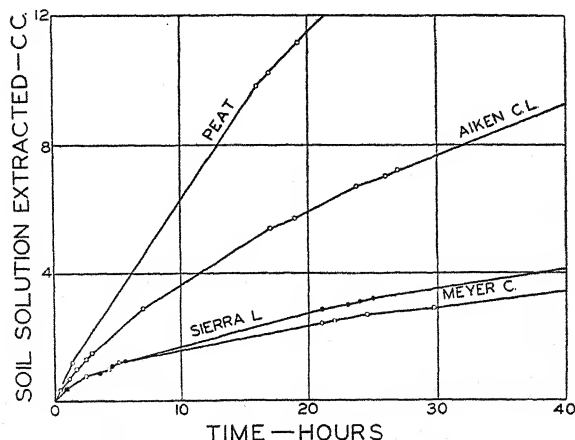


FIG. 3. SUMMATION OF THE SOIL SOLUTION EXTRACTED AS A FUNCTION OF TIME FOR A LAYER OF SOIL 6.3 CM. IN DIAMETER AND 10 CM. DEEP. INITIALLY THE SOIL CONTAINED MOISTURE AT  $\frac{1}{2}$  ATMOSPHERE TENSION AND THE EXTRACTION PRESSURE WAS 6.8 ATMOSPHERES

per square inch above a soft rubber diaphragm which served as the upper gasket for the cell.

It is likely that the soils were somewhat puddled by the handling process. The soils were wetted at zero soil moisture tension and dried to equilibrium at  $\frac{1}{2}$  atmosphere of tension in 6-inch double-walled irrigator pots. The cylinder of the extraction cell was then filled with this moist soil. All soils were handled as nearly as possible in the same way.

The curves illustrate in a general way the rate at which the pressure mem-

brane apparatus will extract moisture from soils containing water at  $\frac{1}{2}$  atmosphere tension. The small cell was used in these experiments; therefore, the extraction rate is only 5 per cent of what it would have been with the large cell.

#### SOIL SOLUTION STUDIES WITH THE PRESSURE-MEMBRANE APPARATUS

Because of several effects, the solution obtained from the pressure-membrane extraction apparatus may differ from the solution that would be delivered to an absorbing root in the same soil. Principal among these may be ion adsorption in the membrane. Since little information on this point is available for Cellophane, the matter will be carefully investigated. For a 1-cm. layer of soil on No. 300 Cellophane, however, the soil-membrane thickness ratio is 450 to 1: therefore trouble is not anticipated in investigating the predominant ions in saline soils.

The 16 atmospheres of gas pressure necessary for low moisture content extractions may produce appreciable changes in the composition of the soil solution because of effects on solubility. The use of nitrogen should prevent large changes in carbonic acid concentration, but where detailed information is wanted on carbonate-bicarbonate relations, it may be necessary to use controlled mixtures of nitrogen and carbon dioxide.

For the experiments here described the liquid and the gas passing through the membrane were trapped in separate vessels by displacing white mineral oil. In some cases the water vapor contained in the gas passing through the membrane may appreciably concentrate the soil solution. This effect can be minimized by humidifying the gas before it enters the extraction chamber. During the 31 hours required for obtaining the Aiken curve in figure 2, about 255 mgm. of water vapor was carried off by the 11 liters of nitrogen that passed through the membrane. This corresponds to 0.03 per cent change in the moisture content of the soil.

In the large cell having a membrane area of 671 sq. cm., about 4 or 5 cc. of solution is required to wet the membrane and fill the spaces in the 80-mesh screen before liquid begins to emerge from the outflow tube.

#### OTHER USES OF THE APPARATUS

The pressure-membrane extraction apparatus seems to provide a means for considerably extending our knowledge of the flow of soil water at moisture tensions greater than 1 atmosphere. Steady-state moisture flow experiments in unsaturated soil can now be carried on over the whole plant growth moisture range and use can be made of well-known energy relations for expressing the results in standard units.

Sorption curves for the whole plant growth moisture range are readily obtainable with this apparatus and will provide useful information on the effect of such treatments as organic matter, fertilizers, salts, puddling, and freezing on the structure and pore size distribution of soils. Applications for the apparatus outside the field of soil science are also apparent.

## SUMMARY

The methods that have been used for extracting solution from soils are briefly discussed under the headings: 1. Displacement, 2. Compaction, 3. Centrifugation, 4. Molecular adsorption, and 5. Suction.

A modified form of suction apparatus is described in which a carefully supported Cellophane membrane serves as the bottom of a gas pressure chamber in which soils are placed for solution extraction. In this way the moisture content of soil in contact with the membrane will be reduced by the amount that would be necessary under normal atmospheric conditions to make the pressure deficiency of the soil water equal to the excess gas pressure in the extraction chamber.

Data are presented for three soils showing that an extraction pressure of 16 atmospheres reduces the moisture content of 5- to 10-mm. layers of soil from saturation to below the wilting point in 24 to 36 hours.

The apparatus can be used for obtaining sorption curves and studying the permeability of unsaturated porous media over a considerably extended range of negative pressure.

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# NOTES ON THE COLORIMETRIC DETERMINATION OF TRACES OF COBALT

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Interest in the cobalt content of soil has been stimulated in recent years by the discovery that cobalt deficiency in forage is responsible for a specific type of malnutrition in the livestock of certain areas, notably in New Zealand and in Florida. Field studies in the latter area have indicated that the soils of the Norfolk series contain very little cobalt,<sup>1</sup> and these studies are being extended in an effort to correlate this deficiency with the soil series. Methods for the determination of cobalt which are described in this paper will, it is hoped, be of immediate practical interest to those who are investigating the content of this element in the soils and vegetation of such areas.

In this investigation, an attempt was made to apply the  $\alpha$ -nitroso- $\beta$ -naphthol color reaction to the quantitative estimation of traces of cobalt. The reagent used was a 0.05 per cent solution of  $\alpha$ -nitroso- $\beta$ -naphthol in very dilute sodium hydroxide. Originally, color was developed by the addition of 10 drops (0.3 ml.) of this reagent to 15 ml. of solution containing amounts of cobalt of the order  $4\gamma$  ( $4 \times 10^{-6}$  gm.), followed by dilution to 25 ml. Color comparisons with such solutions were always difficult and, unless the cobalt contents were virtually identical, highly inaccurate, on account of the yellow color of the reagent itself. This difficulty was removed as a result of the chance discovery that addition of a sulfite to the solution *after* color development, destroyed the yellow color of the reagent but not the brown color of the cobalt compound.

The depth of the color ultimately produced by a given quantity of cobalt was found to depend on the acidity of the solution in which color was developed. After preliminary experiments, ammonium acetate was selected as a suitable buffer for control of acidity. The pH values produced by the addition of various amounts of *N* HCl to mixtures consisting of (a) 15 ml. distilled water plus 3 ml. 10 per cent ammonium acetate and (b) 15 ml. of slightly acid cobalt solution (15 ml. =  $3.75\gamma$  Co) plus 3 ml. 10 per cent ammonium acetate are shown in figure 1, which also indicates the percentage color intensities obtained by the addition of 10 drops of the  $\alpha$ -nitroso- $\beta$ -naphthol reagent to duplicate solutions of the (b) series and subsequent dilution to 25 ml. It will be noted that the maximum color is developed at a pH of approximately 5.2 but that no serious loss of color intensity takes place if the pH is within the range 5.0-5.3.

<sup>1</sup> Unpublished findings of the Florida Agricultural Experiment Station.

The foregoing considerations led to the adoption of the following method of color development:

To an appropriate amount of cobalt in 15 ml. of solution was added 3 ml. of 10 per cent ammonium acetate solution, and the acidity was then adjusted to about pH 5.2 by the addition of *N* HCl. Color was developed by the addition of 0.3 ml.  $\alpha$ -nitroso- $\beta$ -naphthol reagent followed by 0.3 ml. of 10 per cent  $\text{NaHSO}_3$ , to destroy the color of excess reagent. After the addition of 1 ml. of 1:4 sulfuric acid, the solution was made up to 25 ml., when it was suitable

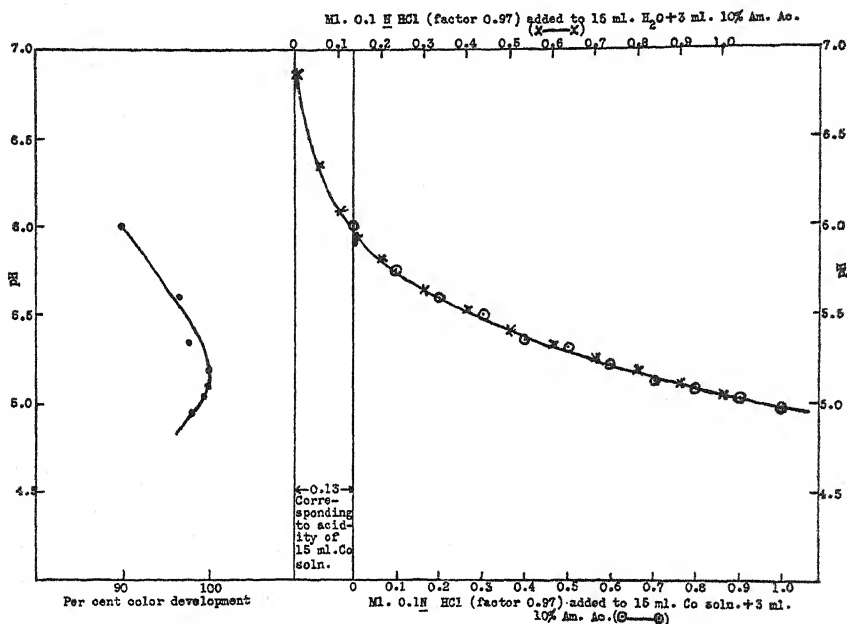


FIG. 1. RELATION BETWEEN pH OF AMMONIUM ACETATE BUFFER AND THE AMOUNT OF ACID ADDED; AND THE INTENSITY OF COLOR DEVELOPED WITH 3.75  $\gamma$  COBALT AT VARIOUS ACIDITIES

for color comparison. The addition of sulfuric acid was found to stabilize the cobalt color, which, without the acid, tended to fade slightly after about half an hour.

#### ADJUSTMENT OF ACIDITY

Provided the cobalt solution is nearly neutral, a pH of 5.2 can be produced by the addition of 0.7 ml. *N* HCl; but as the cobalt solutions involved will usually be more or less acid, methods of dealing with such cases may be noted. When the solution is only slightly acid two methods were found to be satisfactory: (a) the normality of the solution was determined, and a reduction in the amount of *N* HCl was made to compensate for the acidity of the cobalt

solution taken for color development. This method was particularly suitable for the preparation of standards, as the cobalt solutions used were slightly acidified to prevent hydrolysis. (b) The pH of the requisite volume of cobalt solution, plus 3 ml. 10 per cent ammonium acetate, diluted to about 18 ml. was found, and the amount of *N* acid required to change this pH to 5.2 was ascertained by inspection of the graph. When the solutions are more strongly acid, the same methods may be applied, following approximate neutralization with sodium hydroxide. As high concentrations of ammonium salts were found to complicate acidity adjustment, it is advisable to avoid the use of ammonia for neutralization when much free acid is present.

#### REMOVAL OF IRON

As iron produces a color with  $\alpha$ -nitroso- $\beta$ -naphthol, it must be removed from solution. It was found that, with small amounts of iron, this could be achieved by precipitation as basic acetate. When the preliminary neutralization was made with sodium hydroxide, the ammonium acetate was added as a 10 per cent solution, and volumes were suitably adjusted, an aliquot of iron-free solution, equivalent to 3 ml. of 10 per cent ammonium acetate, could be prepared for color development by simply diluting to 18 ml. and adding 0.6 ml. *N* HCl.

#### RESULTS OBTAINED

Four cobalt standards ( $S_1$ ,  $S_2$ ,  $S_3$ , and  $S_4$ ) containing 1.9, 3.8, 5.7, and 7.5 $\gamma$  Co respectively were prepared. In addition, four test solutions containing cobalt and 0.01 per cent Fe were freed from iron by the basic acetate method, and aliquots ( $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$ ) of the resultant solutions were selected to represent corresponding quantities of cobalt. Color was developed in each of these eight solutions by the appropriate methods already described, and comparisons in a Hellige colorimeter resulted in the following estimates of cobalt:

SOLUTION USED AS STANDARD	SOLUTION USED FOR COMPARISON (A)	THEORETICAL Co IN (A)	Co FOUND IN (A)
		<i>gamma</i>	<i>gamma</i>
$S_1$ (1.9 $\gamma$ Co)	$T_1$	1.9	2.1
	$T_2$	3.8	3.5
	$S_2$	3.8	3.6
$S_2$ (3.8 $\gamma$ Co)	$T_2$	3.8	3.8
	$S_1$	1.9	2.0
$S_3$ (5.7 $\gamma$ Co)	$T_3$	5.7	5.6
	$T_4$	7.5	7.0
	$S_4$	7.5	7.4
$S_4$ (7.5 $\gamma$ Co)	$T_4$	7.5	7.2
	$S_3$	5.7	6.1



## CONCLUSIONS

The quantitative colorimetric estimation of traces of cobalt by  $\alpha$ -nitroso- $\beta$ -naphthol appears possible, provided acidity is controlled and the color of excess reagent is destroyed by treatment with sulfite. Color comparisons are valid even between solutions with considerably different cobalt contents. Iron can be removed without difficulty and without impairing the accuracy of the method.

As far as the writer is aware, the use of sulfite for removing the color of the reagent has not been previously reported. It is hoped that the tentative methods described, or modifications of them, may prove of general utility.

# THE AVAILAMETER AND ITS USE IN SOIL MOISTURE CONTROL

## II. CALIBRATION METHODS<sup>1</sup>

R. B. ALLYN AND R. A. WORK<sup>2</sup>

In the preceding part of this paper (1) the availameter was described and its use in routine irrigation practice discussed. In the present paper the calibration and use of the instrument in quantitative studies of irrigation problems, whether in experimental work or on commercial orchards, are shown together with supporting data. The irrigation experiments at the Medford Experiment Station and the soil moisture control project on commercial orchards (3, 4)<sup>3</sup> provided an excellent opportunity to investigate the accuracy of the availameter readings as indicators of soil moisture content.<sup>4</sup>

### SOIL STABILITY VERSUS SOIL MOISTURE

Soil moisture content and soil stability as measured in pounds of load on the double needles of the availameter for the first foot at each of six sampling locations in a 23-acre irrigated Anjou pear orchard on Phoenix clay adobe soil are shown in figure 1. Correlations for the second- and third-foot depths are similar. This illustration was selected because variation of the soil stability-moisture content curves and soil moisture constants between sampling locations in this orchard was more extreme than in most of the other approximately fifty irrigated blocks included in the studies. Actual soil

<sup>1</sup> This paper reports investigations formerly conducted under a cooperative agreement between the Bureaus of Agricultural Engineering and Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station, and more recently conducted under a cooperative agreement between the U. S. Bureau of Plant Industry and the Oregon Agricultural Experiment Station. Presented for publication as Technical Paper No. 339, with the approval of the director, as a contribution of the Medford Branch, Oregon Agricultural Experiment Station.

<sup>2</sup> Assistant Irrigation Engineer, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Associate Irrigation Engineer, Division of Irrigation, Soil Conservation Service, U. S. Department of Agriculture. The authors are greatly indebted to M. R. Lewis, Senior Agricultural Engineer, Soil Conservation Service, for helpful suggestions in the preparation of this manuscript.

<sup>3</sup> Work, R. A. Fourth progress report (1935) on pear irrigation investigations. Unpublished.

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<sup>4</sup> The availameter has been manufactured on order at Medford, Oregon. Inquiries regarding the instrument may be addressed to the Medford Branch of the Oregon Agricultural Experiment Station.

stability determinations and the corresponding moisture contents as determined by oven-drying are shown for each of the six locations by the small plotted symbols.

The widely varying soil conditions found in this orchard would seem to complicate short-cut moisture determination by use of stability measurements. Although good correlation is shown between soil stability and moisture content at each location, it could be concluded that a separate set of conversion factors for each location must be used for determination of moisture conditions in the block. However, if the stability correlation is made with *available* moisture

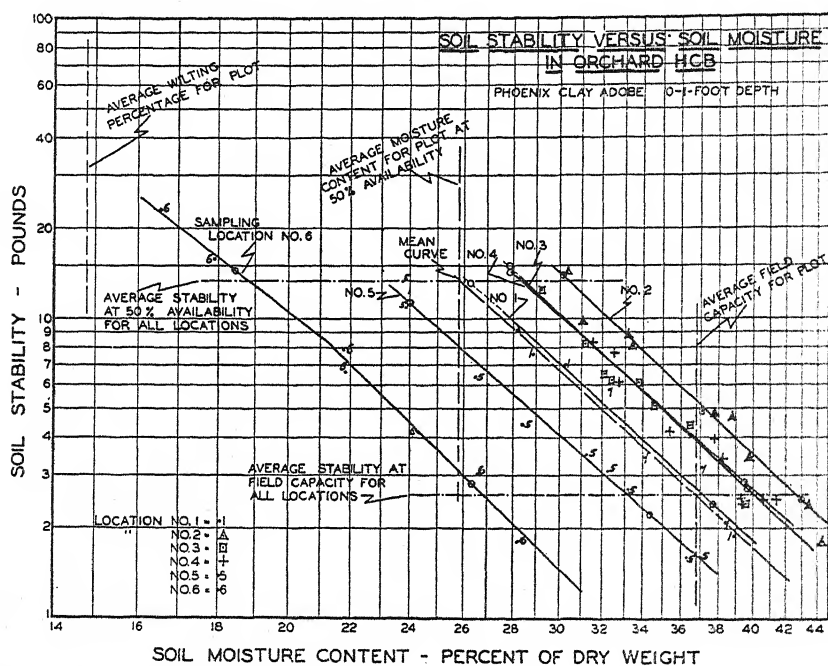


FIG. 1. SOIL STABILITY VERSUS TOTAL SOIL MOISTURE CONTENT IN THE 0-1-FOOT DEPTH IN A COMMERCIAL PEAR ORCHARD NEAR MEDFORD, OREGON (1938)

(expressed as a percentage of the available capacity) rather than with the *total* moisture content used in figure 1, it is found that a single relationship exists even under the extremely variable conditions illustrated.

Field capacity of each foot depth at each sampling location was computed by averaging those moisture determinations made during a 2-year period at times when the soil was judged at field capacity following heavy irrigations and winter rains, as described by Work and Lewis (2). Table 1 gives these values, as well as permanent wilting percentages for each of the sampling locations and for the plot as a whole. Unfortunately, wilting point determinations were made only on samples bulked from all six locations for each foot depth

before the soil variation was realized. The wilting percentage values shown in table 1 for individual locations were computed from the plot average wilting percentage, on the assumption that each bore the same relation to the average as each individual field capacity bore to the average field capacity. This method was based on the well-established wilting point-field capacity relationship (see fig. 6) observed for a number of soils in this locality (2; see also footnote 3 of the present paper).

The field capacity for each location has been plotted on its respective soil stability-moisture content curve in figure 1 as an open circle in the lower part of the figure. The soil stability values at field capacity, in spite of the wide soil variation, all fall between 2.2 and 2.8 with an average of 2.6 pounds.

Open circles indicating the moisture content at 50 per cent available moisture are also plotted on the stability curves of figure 1 near the center of the figure. An average stability of 13.6 pounds substantially represents 50 per cent available moisture at all locations, just as at field capacity all samples irrespective of

TABLE 1  
*Field capacity and wilting percentage on orchard H. C. B.*  
In percentage soil moisture

DEPTH IN FEET.....	FIELD CAPACITY			WILTING PERCENTAGE		
	0-1	1-2	2-3	0-1	1-2	2-3
Location 1.....	37.7	35.2	28.5	15.1	17.1	14.9
Location 2.....	43.0	39.8	35.7	17.3	19.3	18.6
Location 3.....	39.7	36.4	33.3	16.0	17.6	17.4
Location 4.....	39.5	36.0	30.4	15.9	17.4	15.9
Location 5.....	34.3	30.0	37.0	13.8	14.5	14.1
Location 6.....	26.3	28.3		10.6	13.7	
Plot Average.....	36.8	34.3	31.0	14.8	16.6	16.2

location had nearly similar stability in spite of the soil variability. Although the *total* soil moisture at a given soil stability varies widely in the different parts of the orchard, the percentage of *available* soil moisture apparently would be very similar irrespective of location. Replotting the curves of figure 1 on the basis of *available* moisture content using the moisture constants in table 1 results in correlation curves for the individual locations shown in figure 2. All six curves are now thrown closely together, and a given stability determination indicates an approximately similar available moisture condition irrespective of the sampling location or soil variation from point to point. These six separate curves, for all practical purposes, can be represented by one mean curve, also shown in figure 2, and a stability determination at any one of the sampling locations can be converted to moisture availability with reasonable accuracy by use of this mean curve. At the individual locations the determinations may tend to be slightly high or low, but the average of all will closely represent the orchard.

In practice it would be sufficient to take the mean curve from figure 1 and replot this curve in terms of available moisture rather than total moisture content. The resulting curve would be substantially the same as the mean curve of stability versus available moisture shown in figure 2, and is much simpler to construct since it does not require detailed knowledge of the moisture constants at *each* of the sampling locations.

Once such a curve is obtained for each foot of depth, 0-1, 1-2, and 2-3 feet, etc., an avallameter dial reading directly in moisture availability can be pre-

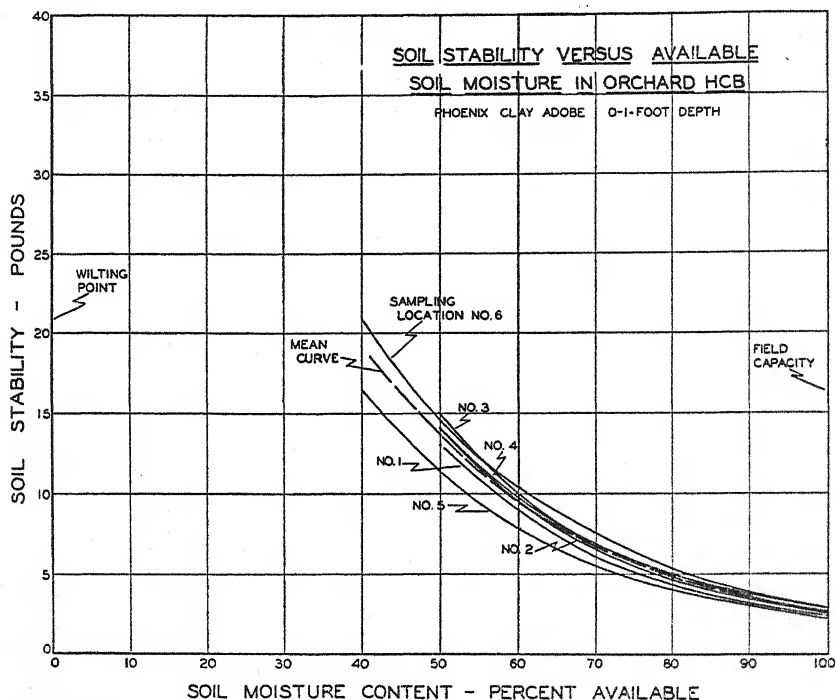


FIG. 2. SOIL STABILITY VERSUS AVAILABLE SOIL MOISTURE IN THE 0-1-FOOT DEPTH IN A COMMERCIAL PEAR ORCHARD NEAR MEDFORD, OREGON (1938)

Same data as in figure 1 except that soil moisture is expressed in per cent available

pared, as shown in figure 3. The dial shown has three scales of available moisture, one for each foot depth. An optional scale in pound units of soil stability is also shown. The dial may be shaded or colored in such a way as to show the operator the soil moisture availability in relation to the need of his crop for moisture. For instance, under average weather conditions for the heavier soils of the Medford area, a moisture availability above 50 to 60 per cent has been found desirable for highest yields of pear trees, and therefore that part of the dial, shown in figure 3, from 60 to 100 per cent available capacity might be colored green to indicate safety; below about 30 per cent

availability, a red shading would indicate moisture deficiency; and the intermediate part might be colored amber, indicating that irrigation water should be applied at the first opportunity, particularly in hot dry weather when transpiration is high.

If the operator desires merely a general picture of the soil moisture condition at any or all of the sampling locations, he notes the respective dial "color" of each sample taken and the preponderance of any particular color value in a series of samples. (It will be noted that the dial illustrated was not prepared for the orchard soil of figure 1.) In case soil moisture conditions are to be

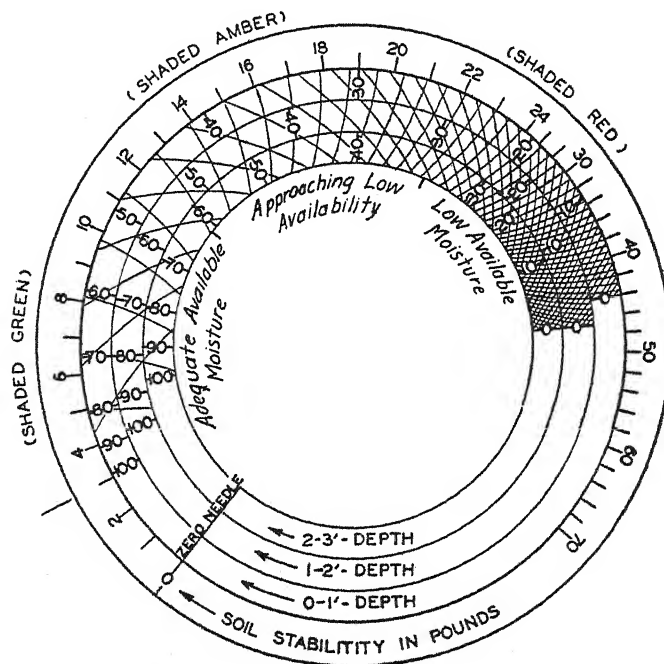


FIG. 3. AVAILAMETER DIAL FOR MAKING SOIL MOISTURE DETERMINATIONS DIRECTLY IN TERMS OF AVAILABLE MOISTURE

This particular calibration and the color zones are for a heavy clay soil (the gauge used in connection has a retarded action above 24 pounds)

followed by the availameter in a number of irrigation blocks having materially different conversion curves, it will be found more practical with the present equipment to make readings in terms of soil stability and convert these to available moisture using conversion curves or tabulated charts prepared for each block rather than interchange dials on the instrument. It may be pointed out here that on some of the orchards having relatively uniform soil included in this study, it was found that soil moisture could be determined in terms of total moisture content equally as simply and accurately as in terms of available moisture.

TABLE 2

Soil stability at various degrees of available soil moisture for a number of Rogue River Valley orchards, 0-1-foot depth

SOIL TYPE	PLOT	FIELD CAPACITY	WILT-ING POINT	SOIL MOISTURE IN PER CENT OF RANGE OF AVAILABLE MOISTURE											
				100	90	80	70	60	50	40	30	20	10	0	
		per cent moisture		pounds											
Clay loam.....	SCE	21.5	10.6	5.2	7.1	9.3	11.9	15.4	20	25	31	40	49	65	
Sites fine sandy loam.....	SSS	21.5	11.1	3.8	4.9	6.8	9.2	13.2	18	24					
Medford gravelly clay loam.....	BCO	21.5	10.5	4.0	5.3	7.3	10.3	13.5	18	23	28	34	41	52	
Sites fine sandy loam.....	SSN	22.7	11.4	3.0	3.9	5.1	6.8	9.6	14	19	26	33	46	60	
Meyer silty clay loam.....	FHHB	22.9	11.5	4.4	6.4	9.0	12.1	15.5	19	23	30	40	53	73	
Medford gravel-ly clay loam..	BCC	22.9	10.3	3.8	5.5	8.2	11.9	16.8	24						
	BCH	23.4	11.8	4.3	5.4	6.9	8.6	11.1	14	19	25	33	39	50	
Sites fine sandy loam.....	FOW	23.7	12.0	3.3	4.4	6.0	8.1	10.8	14	18					
Medford gravelly clay loam.....	BCB 17	24.5	10.3	3.9	5.2	7.2	10.2	14.1							
Meyer silty clay loam.....	FHS	24.6	9.8	2.7	4.1	6.2	9.2	13.7	19	27	39				
Gravelly clay loam.....	VH	25.0	9.8	4.9	6.6	9.5	12.9	17.8	25	31					
Clay loam.....	SCW	26.0	13.4	3.7	5.0	6.8	9.8	13.9							
	FH	27.0	13.4	2.6	3.8	5.2	7.8	11.7	17	23	28	35	43	55	
Meyer silty clay loam.....	FF	27.3	13.6	2.9	4.0	5.6	7.9	11.2	14	17	22	28	37	50	
	MMF	28.7	14.2	3.2	4.3	5.8	8.0	11.2	16						
	MMH	28.9	14.1	3.4	4.4	6.2	7.9	10.7	15						
Meyer clay adobe.....	KE	29.0	13.9	3.6	5.0	7.0	9.9	14.2	19	24	30	38	48	63	
	BBH	29.7	13.0	3.5	5.0	7.2	10.7	15.6	21	26					
Phoenix clay adobe.....	VAB	29.9	12.8	3.3	4.3	5.7	7.5	10.5	15	21	30				
	NOA	31.2	15.3	2.6	3.4	4.6	5.0	7.9	11	15	19	26	34		
	NMC	31.2	15.5	3.1	4.3	5.2	6.6	8.9	12	15	19				
	NMB	31.4	15.4	3.4	4.5	5.4	7.0	9.0	12	16	21				
	NOB	31.4	16.4	3.1	3.9	5.1	6.8	8.4	11	14	17	23	31		
	4 D	31.4	15.0	3.0	3.5	4.3	5.8	8.0	11	15	20	26			
	NOC	31.5	17.1	3.1	4.0	5.0	6.3	8.2	11	14	17	22	28		
	FOE	31.6	14.6	2.6	3.4	4.5	6.5	9.1	13	18					
	FHN	32.1	14.0	3.8	4.8	6.4	8.7	11.2	15						
	NMA	32.3	15.6	3.3	4.2	5.0	7.0	9.3	12	16	21				
Meyer clay adobe.....	4 C	32.7	15.2	3.0	3.5	4.1	5.2	7.1	10	14	19	24			
	FES	32.7	16.5	3.4	4.3	5.5	7.5	9.2	12	16	21				
	NFA	33.1	15.4	3.0	4.1	5.1	6.8	9.2	12	15					
	FEN	33.2	17.2	3.2	4.2	5.3	7.6	9.1	11	15					
	FWN	33.3	15.9	3.2	4.3	5.1	6.3	9.0	12	15	19	26	37		
	FWS	33.8	16.9	3.3	4.2	5.2	6.9	8.5	11	14	19	26	37		
	4 B	33.9	16.7	2.8	3.4	4.6	5.6	7.6	10	13	18				
	NFB	34.3	15.8	2.8	3.8	5.0	6.6	8.2	11	15					
	NFC	34.4	16.0	2.8	3.8	5.1	6.3	8.0	10	14	19				
	FLEN	34.5	17.3	3.3	4.0	4.9	6.1	7.8	10	13	18	26			
	4 A	34.6	16.9	2.9	3.4	4.6	6.0	7.8	10	14	19				
	KW	35.0	14.7	3.0	3.9	5.5	7.6	10.8	15	20	26	34	48		

TABLE 2—*Concluded*

SOIL TYPE	PLOT	FIELD CAPAC- ITY	WILT- ING POINT	SOIL MOISTURE IN PER CENT OF RANGE OF AVAILABLE MOISTURE											
				100	90	80	70	60	50	40	30	20	10	0	
				<i>per cent moisture</i>		<i>pounds</i>									
Aiken clay adobe.	HCR	35.6	17.5	2.6	3.3	4.4	6.0	8.3	12	15					
Meyer clay	FLES	35.8	18.2	3.1	3.9	4.9	6.2	7.4	10	13	18	24			
adobe.....	FLWN	36.0	17.6	3.2	3.9	5.0	6.2	8.1	11	14	19	28			
Aiken clay adobe.	HCB	38.8	15.6	2.7	3.6	4.9	6.9	9.7	14						
Meyer clay															
adobe.....	BCB 7	39.3	17.2	3.1	4.0	5.4	7.0	9.6	13						
Phoenix clay															
adobe.....	VNW	39.4	19.6	2.8	3.6	4.7	6.2	8.4	11	15					
Coker clay adobe.	401-45	39.9	18.4	2.6	3.4	4.4	5.9	8.1	11						
Phoenix clay	VSE	40.4	20.5	2.1	2.7	3.5	4.7	6.2	9	12					
adobe.....	CP	41.7	21.7	1.9	2.4	3.3	4.3	5.9	8	10					
	RA	41.7	21.3	2.0	2.5	3.2	4.3	5.7	8						
Coker clay	401-95W	42.3	17.9	2.9	3.8	5.1	6.9	8.9	12						
adobe.....	401-95E	44.8	18.8	2.8	3.8	5.2	7.5	9.1	12						

## CALIBRATION PROCEDURE

Calibration of the availameter for the soil of a given orchard has consisted of making stability readings with the availameter and moisture content determinations by oven drying of samples taken at selected sampling locations over a period of time so as to include the widest possible range of soil moisture. Over a 2-year period, approximately 50,000 determinations of soil stability versus moisture content have been made on approximately fifty blocks of irrigated pear orchards scattered over an area of 40 square miles in the upper Rogue River Valley. The individual blocks varied from 1 to 40 acres. Ten distinct soil types were represented with field capacities ranging from 19 to 45 per cent. Table 2 summarizes the major part of these data for the 0-1-foot depth, showing the soil stability for different degrees of available moisture between field capacity and wilting percentage. Space does not permit inclusion of data for 1-2- and 2-3-foot depths. Generally, however, stability increases somewhat with depth for a given available moisture content. In figure 4 data on the 0-1-foot level in many of these orchards are shown in which soil stability is plotted against total moisture content. Each curve represents the average of three to six sampling locations, depending on the size of each orchard, as do the data shown in table 2.

Replotting the data of figure 4 in terms of percentage of the available capacity for each soil results in a much more compact group of curves similar to those in figure 2. Since soil moisture in these orchards was kept fairly highly available by irrigation, samples in the lower range of available moisture could not be obtained directly in the field for calibration. As a part of the calibration study, therefore, sets of sample cores of a few soils were taken in



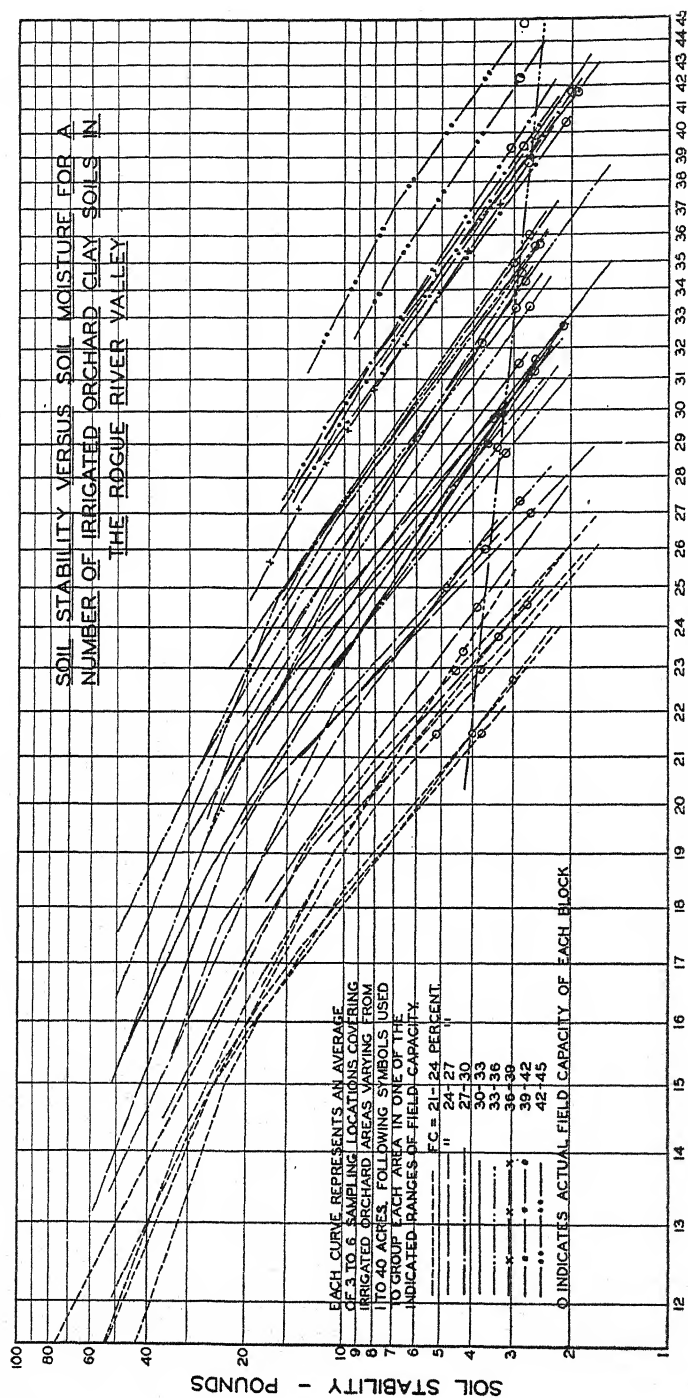


FIG. 4. SOIL STABILITY VERSUS SOIL MOISTURE IN THE 0-1-FOOT DEPTH FOR A NUMBER OF IRRIGATED ORCHARD CLAY SOILS IN THE ROGUE RIVER VALLEY, OREGON (1938)

the field and, by air-drying each set for different periods of time, the part of the calibration curves covering the lower moisture contents were completed. These determinations appear to compare favorably with those made directly from samples taken in the field. As it is possible that changes in consolidation of some soils during the air-drying process may be a factor in making a correlation by this process, studies are contemplated in which several sets of samples, taken when soil moisture conditions are near field capacity, will be exposed to different periods of air-drying under controlled humidity. Stability and oven moisture determinations on such samples may prove to be a means for quickly preparing a complete calibration.

An examination of figure 4 shows that the stability-moisture curves range across the chart in order from light to heavier soils as in figure 1. The slopes of a few of the curves vary somewhat from the group, perhaps partly because of the limited amount of calibration data obtained on some of them. The open circles indicate the soil stability at field capacity for each curve. The field capacity-stability trend from lighter to heavier soils is shown by the line passing somewhat downward to the right through the group of circles. Average control curves computed from figure 4 are shown in figure 5. To use this chart for calculating an approximate availameter calibration, it is first necessary either to determine or to estimate the average field capacity of the orchard block in question or to make one initial stability-moisture content determination for each foot depth at each of the sampling locations selected. In case the field capacity value is known, a curve can be interpolated between those shown on the chart, starting at the field capacity value on the field capacity line and proceeding either graphically or by means of the formula shown.

The graphical procedure is much more convenient and certainly gives all the accuracy that is justified by this approximate method. If the formula is used, however, it should be noted that each equation that is set up applies only to one straight section of the curve. Every curve changes shape at the intersection with the dashed lines, and the coordinates of this intersection are used as values of  $A$  and  $B$  in proceeding with the next adjacent straight section. Values of exponent  $S$  may be interpolated from the values shown.

If one or two initial stability-oven moisture content determinations for each location in the sampling area are plotted on the chart, an approximate curve can be drawn passing through the average of these plotted points and paralleling the adjacent control curves. The approximate field capacity will be the intersection with the field capacity line.

It may be desirable that the approximate curve of soil stability versus total moisture content thus obtained be converted to a soil stability versus available moisture content curve for the best results at the individual sampling locations. A value for the wilting percentage of the soil is needed for this conversion. In the Medford area the permanent wilting percentage can be estimated from the field capacity by the use of the curves in figure 6 showing the relation of these constants for a number of local soils. It is desirable that the estimated

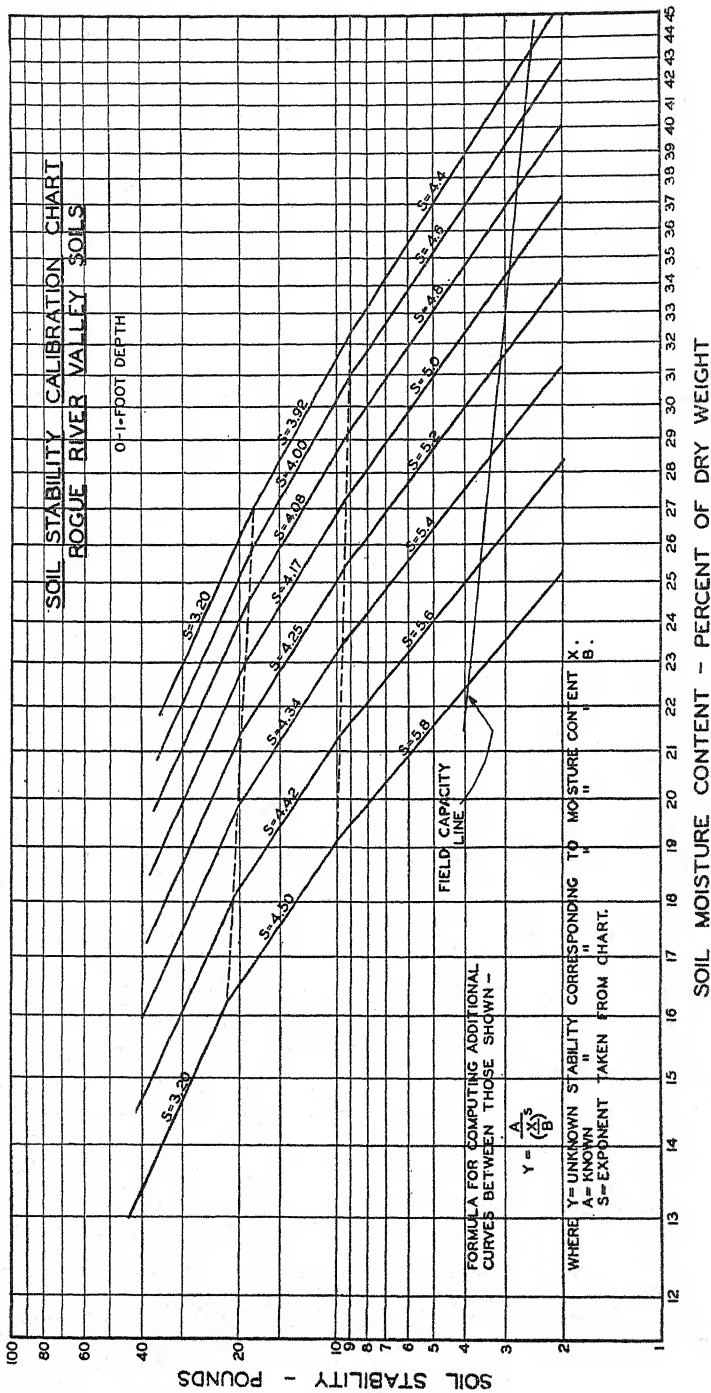


FIG. 5. SOIL STABILITY-SOIL MOISTURE CONTENT CALIBRATION CHART FOR ROGUE RIVER VALLEY SOILS, OREGON, FOR THE 0-1-FOOT DEPTH

values of both field capacity and permanent wilting percentage as well as the stability-soil moisture relation be checked by actual determination when convenient. Checking of the stability-moisture relation is best accomplished by plotting the percentage available moisture as determined by the oven method against that determined by the availameter for a number of different moisture

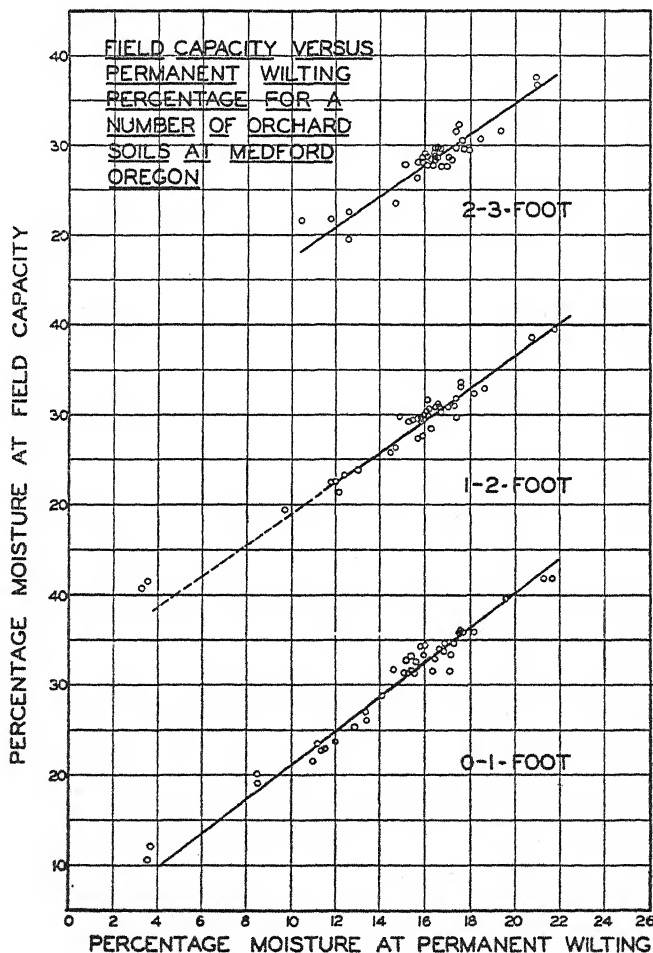


FIG. 6. FIELD CAPACITY VERSUS PERMANENT WILTING PERCENTAGE FOR A NUMBER OF ORCHARD SOILS NEAR MEDFORD, OREGON

conditions. By noting the deviation of the resulting curve trace from a curve of true correlation, the necessary shifting of the assumed curve can be determined.

Tables 3 and 4 show a comparison of results obtained by the availameter method and the standard oven method for a number of different orchard

TABLE 3

*Comparison of available soil moisture as determined by standard oven method and by the availometer*

DATE	SOIL DEPTH											
	0-1 foot			1-2 feet			2-3 feet			0-3 feet		
	Per cent of Available Moisture											
	Oven	Availa- meter	Diff.	Oven	Availa- meter	Diff.	Oven	Availa- meter	Diff.	Oven	Availa- meter	Diff.
Plot FLWSN 0-3' FC = 33.4%, 0-3' WP = 17.6%, Meyer clay adobe												
1939												
3-1	91	92	1	93	93	0	99	96	3	94	94	0
4-4	68	68	0	88	86	2	96	95	1	84	84	0
4-21	42	42	0	59	61	2	80	81	1	60	61	1
5-11	27	26	1	38	38	0	53	53	0	39	39	0
5-27	31	29	2	32	31	1	45	42	3	36	34	2
6-21	62	62	0	69	72	3	63	63	0	64	66	2
7-6	29	29	0	34	36	2	39	39	0	34	35	1
7-26	45	46	1	46	47	1	39	39	0	43	43	0
8-14	45	45	0	49	49	0	41	41	0	45	45	0
8-22	75	73	2	46	46	0	34	35	1	52	51	1
9-1	49	49	0	52	51	1	39	39	0	47	46	1
9-13	19	19	0	23	24	1	24	25	1	22	23	1
Average Difference.....			0.6			1.1			0.8			0.7
Plot B-4B 0-3' FC = 31.7%, 0-3' WP = 16.5%, Meyer clay adobe												
1939												
3-28	72	77	5	83	87	4	91	94	3	82	86	4
4-19	84	85	1	88	86	2	87	86	1	86	86	0
5-4	60	64	4	73	77	4	88	90	2	74	77	3
6-14	44	48	4	45	47	2	47	48	1	45	48	3
6-29	60	56	4	56	52	4	55	51	4	57	53	4
7-31	32	32	0	41	40	1	39	38	1	37	37	0
8-21	38	38	0	37	37	0	38	39	1	38	38	0
8-26	82	79	3	65	63	2	63	61	2	70	68	2
9-7	38	38	0	37	35	2	35	34	1	37	36	1
Average Difference.....			2.3			2.3			1.8			1.9
Plot FHS 0-3' FC = 27.4%, 0-3' WP = 12.5%, Meyer silty clay loam												
1938												
4-4	98	101	3	99	100	1	98	99	1	98	100	2
5-11	67	67	0	93	89	4	103	98	5	88	85	3
5-26	52	57	5	78	79	1	82	83	1	71	73	2
6-7	74	72	2	77	79	2	74	76	2	75	76	1
8-9	54	55	1	59	62	3	54	56	2	56	58	2
8-24	38	36	2	48	49	1	48	45	3	44	43	1
12-6	87	91	4	71	73	2	58	58	0	72	74	2
Average Difference.....			2.4			2.0			2.0			1.9

blocks and soils in 1938 and 1939. The result in each case is an average from samples taken at three to five sampling locations, depending on the size of the block. The availameter determinations were made on soil samples in the field, and these same samples were then brought to the laboratory in sealed containers for oven moisture determination. These tables include all availameter determinations made on each of these orchards during the season.

TABLE 4

*Comparison of total soil moisture content as determined by standard oven method and by the availameter*

DATE	SOIL DEPTH											
	0-1 foot			1-2 feet			2-3 feet			0-3 feet		
	Total Moisture Content in Per cent											
	Oven	Availa- meter	Diff.	Oven	Availa- meter	Diff.	Oven	Availa- meter	Diff.	Oven	Availa- meter	Diff.
<i>Plot BCO 0-3' FC = 23.3% approx., 0-3' WP = 11.8%, Medford gravelly clay loam</i>												
1938												
4-14	20.2	20.4	.2	22.3	22.2	.1	23.7	23.6	.1	22.1	22.1	0
4-21	19.9	20.2	.3	22.5	23.1	.6	22.8	23.4	.6	21.7	22.2	.5
5-4	20.1	20.8	.7	21.0	21.4	.4	22.1	22.3	.2	21.1	21.5	.4
5-18	18.7	19.3	.6	20.5	21.2	.7	21.5	22.1	.6	20.2	20.9	.7
6-6	21.5	21.2	.3	25.0	24.5	.5	23.2	23.0	.2	23.2	22.9	.3
6-22	17.2	17.8	.6	19.8	20.3	.5	21.2	21.4	.2	19.4	19.8	.4
6-27	14.3	13.9	.4	16.8	16.7	.1	17.4	17.2	.2	16.2	16.0	.2
8-27	18.4	18.8	.4	21.2	21.5	.3	22.5	23.1	.6	20.7	21.1	.4
12-7	20.4	20.2	.2	21.1	20.7	.4	20.4	20.7	.3	20.6	20.5	.1
Average Difference.....			.4			.4			.3			.3
<i>Plot GCH 0-3' FC = 22.6% approx., 0-3' WP = 11.6%, Medford gravelly clay loam</i>												
1938												
4-14	23.4	23.6	.2	22.9	22.9	0.0	21.6	21.9	.3	22.6	22.8	.2
4-21	21.3	21.2	.1	22.1	21.9	0.2	21.9	22.1	.2	21.8	21.7	.1
5-4	21.1	21.4	.3	22.1	22.5	0.4	20.9	21.6	.7	21.4	21.8	.4
5-18	18.8	18.4	.4	21.4	20.4	1.0	21.1	20.5	.6	20.4	19.8	.6
6-10	20.1	20.7	.6	22.2	22.2	0.0	23.3	23.4	.1	21.9	22.1	.2
7-7	21.7	20.9	.8	21.4	20.6	0.8	21.7	20.9	.8	21.6	20.8	.8
8-18	20.3	20.5	.2	21.4	21.5	0.1	22.3	22.6	.3	21.3	21.5	.2
12-8	21.8	21.1	.7	23.9	23.7	0.2	22.9	22.8	.1	22.9	22.5	.4
Average Difference.....			.4			0.3			.4			.4

In table 3 it should be noted that the maximum difference between available moisture determined by the oven and by the availameter was as great as 5 per cent of the available capacity in only three cases of the 84 single-foot depths shown and was within 2 per cent in over three-fourths of the tests. The 0-3-foot average determination shows even better accuracy than this.

In table 4, showing total soil moisture content, the difference was as great

as 1 per cent in only one case of the 51 individual-foot depths shown and was less than 0.5 per cent in nearly three-fourths of the tests. This very close agreement between results of the two methods is considered excellent proof of the avallameter accuracy.

Form 6										Page _____	
Hole No.	Depth Sample	Soil Stability by Avallameter				Available Moisture in percent				Average Avail-ability	Remarks
1	0-1	16.1	14.9	19.7	19.8	38	40	30	30	34	7.5' from tree
	1-2	15.9	19.7	17.8	19.6	40	31	35	31	34	E-11
	2-3	18.2	16.0	14.8	12.9	38	44	47	52	45	
2	0-1	12.0	10.6	13.8	18.0	48	53	43	33	44	4.5' from tree
	1-2	13.3	13.4	15.7	18.0	48	48	40	34	42	J-12
	2-3	14.0	10.7	15.6	12.2	49	60	44	55	52	
3	0-1	19.5	15.8	15.5	15.1	30	39	38	40	37	13.0' from tree
	1-2	13.4	10.9	12.8	13.6	48	57	50	47	50	I-13
	2-3	14.4	14.1	15.7	16.6	48	49	44	42	46	
4	0-1	17.3	18.9	18.2	14.1	35	32	33	42	35	12.0' from tree
	1-2	16.3	16.0	18.2	17.5	39	40	34	36	37	E-12
	2-3	11.8	16.8	16.1	13.5	56	42	44	50	48	
5	0-1	15.3	14.5	17.6	13.3	40	42	34	45	40	10.5' from tree
	1-2	15.0	13.5	16.4	13.5	42	47	38	47	43	E-13
	2-3	15.6	16.8	14.1	13.7	44	42	49	50	46	
						PLOT AVERAGES					
						0-1' = 38					
						1-2' = 41					
						2-3' = 47					
						0-3' = 42					

Determinations by J. M.

Calc. by C. F. R.

Orchard F

Date taken - 7-26-39

Checked C. F. R.

Plot No. N.

FIG. 7. TABULAR FORM USED IN THE FIELD FOR RECORDING SOIL STABILITY OR AVAILABLE SOIL MOISTURE DETERMINATIONS AS MADE BY THE AVALLAMETER

The form shown in figure 7 is used to record avallameter data in the field. If a dial reading directly in terms of available moisture has been prepared, the determinations on each of the four short  $2\frac{1}{4}$ -inch cores (the width of the sample holder) used from each 1-foot sample are directly recorded and averaged. If soil stability only is being measured by the instrument, then that is

recorded for each small core of each sample. These determinations are individually converted to available moisture by means of conversion curves previously developed, and the average is computed for the 1-foot sample. Variations in stability more pronounced than those indicated in figure 7 and corresponding variations in moisture content of the short cores comprising the 1-foot sample are usual. Each stability determination must be individually converted to its corresponding moisture content because the relationship between soil stability and soil moisture is not linear. This can be illustrated by a hypothetical core consisting of two parts with widely divergent stabilities of, say, 5 and 15 pounds. Using the mean curve of figure 2, the average of these stabilities, 10 pounds, is equivalent to 58 per cent available moisture. The equivalent moisture availabilities of each of the two stability determinations, 80 per cent for 5 pounds and 47 per cent for 15 pounds, average 63 per cent. The second method is the proper one; use of the first would indicate a moisture availability 5 per cent too low.

In our earlier work on the calibration of the instrument, oven moisture determinations were made on each  $2\frac{1}{4}$ -inch core and plotted against the corresponding stability readings. This method required considerable precision in weighing the small cores. Moreover, the calibration was carried on in conjunction with regular soil moisture work in which the average moisture content of a great many 1-foot cores was needed. It was found that labor could be considerably reduced by making the oven determinations on whole 1-foot samples (consisting of four short cores) and plotting the results against the average stability (of the four cores) minus a small correction.

In the foregoing illustration, the sample contained an average of 63 per cent available moisture as determined by use of the mean curve of figure 2. This curve also shows that 63 per cent available moisture corresponds to a stability of 8 pounds. The average of the stabilities of the two parts, 10 pounds, could be plotted against the moisture content of 63 per cent when making a calibration curve, if a correction of 2 pounds were deducted from the average; that is,  $10 - 2 = 8$  pounds. Corrections of the average can be computed in this way for any combination of stability readings. Any assumed curve similar to the mean of figure 2 is sufficiently accurate for this purpose. These stability corrections were computed for a large number of assumed conditions and plotted in the form of charts, so that they could be rapidly applied to the data. The number of  $2\frac{1}{4}$ -inch cores in the sample unit, the arrangement of the readings, and the maximum spread of the stability readings encountered among the several short cores of the sample appeared to be the main factors determining the amount of correction. Generally, if the stability spread is no greater than one-half of the average, the correction can be disregarded.

Now that the shape of a stability-moisture content curve for a given soil can be generalized fairly accurately (see fig. 5), however, it is much simpler, as previously outlined, to assume this curve temporarily in making the conversions. Oven moisture determinations made on the same samples will



indicate how much the assumed curve should be shifted for closer agreement. In this way the use of the correction factors can be avoided entirely.

#### SUMMARY

Calibration and use of the availameter in quantitative soil moisture studies are shown. In the direct calibration method, soil stability measurements by the instrument were compared with oven-dry soil moisture determinations over a wide range both of soils and of moisture content. The correlation curves thus obtained can be converted into terms of available soil moisture by the use of soil moisture constants. A good correlation between availameter soil stability readings and available soil moisture is shown even where soil moisture constants varied somewhat from location to location in small areas. A method whereby an approximate calibration may be quickly prepared from limited local data is also presented.

Comparisons of soil moisture as determined by oven-drying and by the availameter showed close agreement. The difference was less than 2 per cent of the available soil moisture capacity in the great majority of cases on heavy clay soil. Comparisons in terms of total moisture content showed differences of less than 0.5 per cent in most cases. Very little time additional to that required to obtain the field samples alone is needed to complete the moisture determinations by the availameter method. The complete operation is made directly in the field without technical skill or laboratory equipment.

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# THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XXIII. THE CONSTITUTION OF THE PEDOSPHERE AND SOIL CLASSIFICATION

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Soils, like other bodies, must be defined and classified on the basis of their characteristics, as pointed out by Marbut (13) and by de'Sigmond (26), and not on assumed external soil-forming factors, such as geology and climate. But soils cannot be classified like plants and animals, which exist as *species* and between which there are no gradations. Soils form *series* showing all gradations from one type to the other. They do not form a mosaic on the surface of the earth but represent instead the continuous variation in the composition and morphology of the pedosphere. Soils are parts of the pedosphere much like the tissues of an organism, some differentiated, some undifferentiated. Soils, therefore, should not be treated as independent bodies but as morphological parts of a larger body, the pedosphere. The great soil groups of Glinka (7) might be looked upon, from this point of view, as the anatomical formations or organs of this body.

By an extension of this biological analogy, we get the following division:

The body = the pedosphere.

The anatomy = the great soil groups.

The morphology = the soil type as based on profile characteristics.

The histology = the textural units.

The protoplasm = the colloidal complex.

The physiology = the soil solution.

## THE PEDOSPHERE

Soils might be said to represent the sum of the mechanical and the products of the chemical interaction of the four spheres: the lithosphere, the hydrosphere, the atmosphere, and the biosphere. They constitute a dispersed system in which material from these four spheres alternate as dispersed phase and dispersion medium. To this sphere of spheres the name "pedosphere" has been given.

The constitution of the pedosphere will vary with the proportions and the intensity of action of the different spheres which contribute to its formation. The great variation of soils, of which no two are exactly alike, is an expression of this variation in the activity or dominance of the different spheres. Thus we have the mineral soils in which the lithosphere dominates, the organic soils

in which the biosphere dominates, the ground-water soils in which the hydrosphere dominates, and the dry porous soils in which the atmosphere might be said to dominate.

Besides these four-sphere systems we meet with three-sphere and two-sphere systems in which one or two of the spheres are absent. We have, in other words, dispersed systems representing every combination of the four spheres.

The following examples of the different systems might be given:

LA = lithosphere + atmosphere systems. This is found in the barren desert where the dispersed system consists of mineral particles and air. Here is neither water nor life.

AB = atmosphere + biosphere systems. The uppermost part of the soil profile consisting of the plant cover or the aerial dispersion of pollen might be given as an example.

HB = hydrosphere + biosphere systems. A pond with its countless organisms.

LH = lithosphere + hydrosphere systems. Waterlogged sand or clay under sterile conditions forms such a system.

LAH = lithosphere + atmosphere + hydrosphere systems. A system of earth, water, and air but no life indicates sterile conditions such as the extreme types of solonchak (saline soils).

LAB = lithosphere + atmosphere + biosphere systems. There can be no life without water, but the remains left in dry places by migratory forms of life may result in such systems, of which the guano deposits are examples.

HAB = hydrosphere + atmosphere + biosphere systems. The organic soils and forest litter form such systems.

LHB = lithosphere + hydrosphere + biosphere systems. Waterlogged soils and lake bottoms are examples.

Finally we have the four-sphere systems LAHB of which there are infinite varieties.

The fundamental factors of composition of a soil are those usually expressed as air-space, moisture, organic matter, and ash. These factors express the proportions, in terms of volume and weight, in which the atmosphere, the hydrosphere, the biosphere, and the lithosphere respectively, enter into the make-up of the soil. In terms of volume the composition of the pedosphere might therefore be expressed as follows:

$$L + A + H + B = 100$$

where the symbols represent the percentage volume of the component spheres.

#### THE ANATOMY OF THE PEDOSPHERE—THE GREAT SOIL GROUPS

If we imagine the earth stripped of all vegetation and plowed and harrowed, and ourselves, on a cloudless day, transported to a point a few thousand miles above the equator, we shall obtain a picture of the anatomy of the pedosphere as here conceived. The great soil groups would reveal themselves by their color, which, in soils, is not a mere pigment but an expression of constitutional differences as these have resulted from the interplay of the lithosphere, the atmosphere, the hydrosphere, and the biosphere. The anatomy of the pedosphere is, therefore, based on these major components.

Now, soils might be grouped according to the dominance of L, A, H, and B, but since no sharp lines could be drawn between such groups, a division of that

kind would lead to confusion and would be, at best, very complicated. A division must be simple and must be based upon as few factors as possible and ones which allow a quantitative measurement.

Among the four spheres which contribute to the formation of the pedosphere, the hydrosphere is the most dynamic. This is, of course, due to the mobility of water, which undergoes cyclic changes from one to the other of the three states of matter, the solid, the liquid, and the gaseous. In the cyclic movement of water great changes are wrought in the soil in the form of eluviation and illuviation, and it is for this reason that the influence of the hydrosphere forms the basis of most systems of soil classification. The degree of leaching and the height of the ground-water level are intimately related to most of the fundamental characters of a soil, among which the base status and the humus content are the most important.

But we cannot, of course, classify soils on the basis of their water content, which together with the air space varies in a complementary way from day to day. The hydrospheric and the atmospheric components of the pedosphere are both too variable to serve as a basis for classification.

The components of the lithosphere and the biosphere, which as percentage residue and loss on ignition, respectively, give the solid materials of the soil, are much less subject to fluctuations in a given soil. These components have, therefore, rightly served as a basis in some of the earlier systems of classification based on soil characteristics. We shall here express the material constitution of the pedosphere by the organic matter (humus) content which, in terms of percentage, also gives the percentage of mineral matter as its complement.

Having found an expression for the fundamental differences in the *composition* of the soil material, we must next aim to find an expression for the most important *condition* of that material. In the writer's opinion, this condition is found in the base status. It is on these two factors then that the proposed system of group classification is based. This gives us a "two-dimensional" system, within which we shall find a place for every soil.

#### *The humus content*

In figure 114 the soils are charted on the basis of their percentage humus content and base status. With respect to the humus content the soils might be divided into three main groups as follows:

I. Lithopeds	<1 per cent organic matter
II. Humo-lithopeds	1-12 per cent organic matter
III. Humopeds	>12 per cent organic matter

Though this division rests, to some extent, on a hydrogenic basis, it does not allow any natural lines to be drawn and is, therefore, not sharp. Some division of this kind is necessary, however, in order to group the soils on the basis of their major components.

### The base status

On the basis of the base status the soils naturally divide themselves into three main groups as follows (19):

- A. The "supersaturated" or alkaline soils
- B. The partly saturated or semisaturated soils
- C. The negatively base-saturated or acid-saturated soils

By this division we go a step beyond Marbut's pedocals and pedalfer (13), Robinson's unleached and leached soils (25), and Gedroiz's Na-, Ca-, and H-

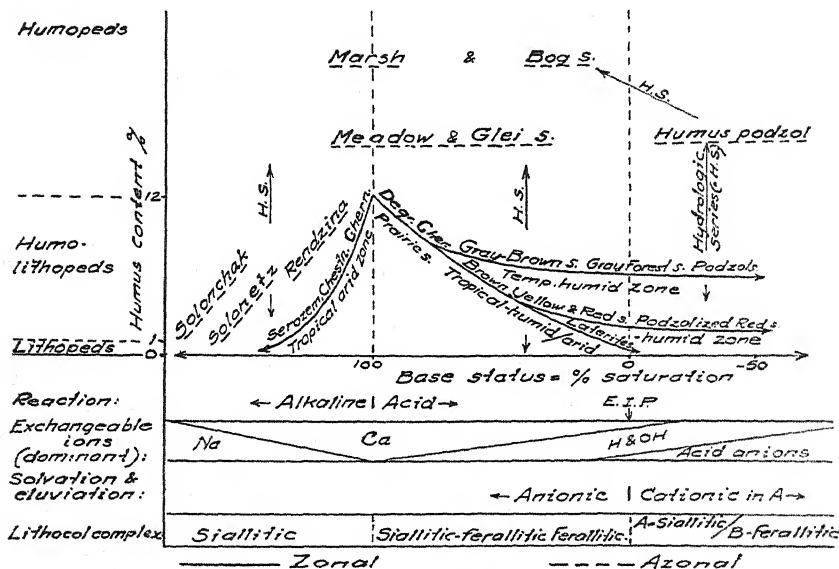


FIG. 114. A CLASSIFICATION OF SOILS ON THE BASIS OF HUMUS CONTENT AND BASE STATUS

soils (6), in that we draw a line between the nonpodzolized and the podzolized soils which are morphologically and chemically very different.

The above division gives us, then, nine subgroups as follows:

- A I. Supersaturated lithopeds
- B I. Semisaturated lithopeds
- C I. Negatively saturated lithopeds
- A II. Supersaturated humo-lithopeds
- B II. Semisaturated humo-lithopeds
- C II. Negatively saturated humo-lithopeds
- A III. Supersaturated humopeds
- B III. Semisaturated humopeds
- C III. Negatively saturated humopeds

If we consider a neutral soil to be 100 per cent saturated, then a soil which is alkaline and contains carbonates and in many instances considerable amounts of free salts must be looked upon as supersaturated. In the presence of salts, as in unleached soils, the capacity to bind base is greatly enhanced. A solonchak liberates a great amount of base when leached of its salts and is thus converted into a solonetz through the formation of sodium carbonate.

The negative base saturation expresses that condition of a soil in which it contains a greater amount of combined acids than combined bases. The base status of a soil must be expressed by the percentage *net* base saturation. Thus at zero saturation the soil contains equal amounts of combined acid and base. Only a completely dialyzed soil can be absolutely unsaturated. Such a soil probably does not exist in nature.

How far the completely leached soils, such as the podzols and the laterites, become saturated with acids in excess of bases has never been determined, but that acid anions can be present in excess of metal cations is an established fact. Thus a B<sub>2</sub> sample of the Haggbygget podzol yielded 1.6 m.e. SO<sub>4</sub> ions per 100 gm. when treated on the steam bath first with ammonia and then with NH<sub>4</sub>Cl solution. The total electro dialyzable bases of the same samples amounted to only 0.3 m.e. The Las Mesas laterite (22) showed much greater differences. With the same treatment, the four samples (I = 0-12 inches, II = 12-23 inches, III = 26-38 inches, and IV = 43-58 inches) from the surface down to 58 inches yielded 2.6, 6.8, 4.7, and 4.6 m.e. SO<sub>4</sub>/100 gm. respectively. The surface concretions gave 4.7 m.e./100 gm., whereas the parent material of serpentine rock showed no SO<sub>4</sub>, whether by displacement or by fusion analysis. The laterite yielded mere traces of electro dialyzable bases. The sulfate ions are held so tightly that they are readily displaced only by the OH ions (probably also by PO<sub>4</sub>). That the sesquioxides which precipitate in the B horizon of the podzol profile are more or less saturated with acids is not surprising, for having arrived there in the form of a cationic (acid-saturated) sol complex, they could not be otherwise. Nor is it surprising that the highly desilicated and strongly basoidal laterites fix more anions than cations from the percolating soil solution.

The base status can be determined by the following method:

1. The soil is electro dialyzed until completely desaturated. Saline soils containing chlorides should first be washed free from Cl, which otherwise dissolves the anode metal. The electro dialyzed soil is electrometrically titrated to pH 7 by standard calcium hydroxide. This gives the capacity  $C$  of the soil to bind Ca at pH 7.
2. The original soil is titrated to pH 7 in the same manner. This gives the total acidity  $H$ , which represents the sum of the exchangeable hydrogen ions (the free acidoids) and the free and the exchangeable acids. If the soil is alkaline, i.e., supersaturated, it will contain an amount of base  $b$  in excess of  $C$ . Because of the presence of carbonates, this base cannot be found by back titration. For want of a better method, therefore, the following may be used. The soil is washed practically free from salts, and the pH is determined. The amount of base that the soil will bind at this pH is then read off from the titration curve of the electro dialyzed soil. This amount gives  $C + b$ .

Now, the *net* amount of base (base - acid) in an unsaturated soil is  $C - H$ . This difference becomes negative in the negatively base-saturated soil where  $H > C$ . The net percentage base saturation or the base status thus becomes

$$1 \quad \frac{C + b}{C} \cdot 100 = > 100 \text{ in supersaturated soils;}$$

$$2 \quad \frac{C - H}{C} \cdot 100 = 0-100 \text{ in semisaturated soils; and}$$

$$3 \quad \frac{C - H}{C} \cdot 100 = < 0 \text{ in negatively saturated soils.}$$

### *Solvation and eluviation*

All leached soils fall, further, into two natural groups as follows (18):

1. Anionically solvated and eluviated.
2. Cationically solvated and eluviated.

*Anionic solvation and eluviation.* As long as a soil possesses a positive base status, the primary silicates and the gel complex undergo a basic hydrolysis during which ionized acidoid groups split off and become solvated in association with the exchangeable cations. A movement of the soil solution results then in an eluviation of acidoids (upward or downward) in the form of an anionic sol complex, thus leaving a lithocol gel complex which is richer in basoids (chiefly sesquioxides) and poorer in acidoids (chiefly silica).

Since the pH of a soil normally increases downward, the anionic sol complex cannot be precipitated in the lower horizons where its ionization must, on the contrary, increase. Nor can this complex, which is extremely highly dispersed, be caught by infiltration as in the case of the deflocculated primary soil particles. Anionically eluviated soils show, therefore, a fairly uniform composition of the colloidal fraction throughout the profile.

Many soils, however, which belong to the anionically eluviated group such as degraded chernozem, prairie soils, and the gray-brown soils show an increase in sesquioxides with depth. These soils are therefore said to be podzolized. Since these soils often possess a relatively high base status which, together with a high content of humus acidoids, precludes a cationic eluviation (podzolization), this conclusion is, in the writer's opinion, erroneous. It must be remembered that the smallest particles which possess the greatest surface have been subjected to a greater anionic solvation and are therefore richer in sesquioxides than the larger particles. Now when the leaching has reduced the free electrolytes and the exchangeable Ca to a certain critical point the aggregates will deflocculate and the smallest particles will be washed down, either to become reflocculated or to be infiltrated in a lower horizon. It is therefore necessary that we distinguish between physical deflocculation and chemical solvation and the changes brought about by these processes.

The extent to which soils become anionically eluviated by the time they reach the zero position with respect to the base status will depend on the pre-

vailing pH. The sesquioxides combine with, and precipitate isoelectrically, more silica in an acid than in a neutral medium. Ferric and aluminum hydroxides are isoelectric at about pH 7 and 8 respectively and bind no silica above these points (15).

Where the weathering is intensive and where there is but a slight accumulation of humus acidoids, as in the humid tropics, a relatively high pH will prevail, and the leaching of the bases will be accompanied by an extensive solvation and eluviation of the silica. This will therefore result in the formation of ferallitic soils (cf. tropical humid branch in figure 114).

In the colder regions where the chemical weathering is much slower and where there is an accumulation of humus acidoids, the pH will be lower and

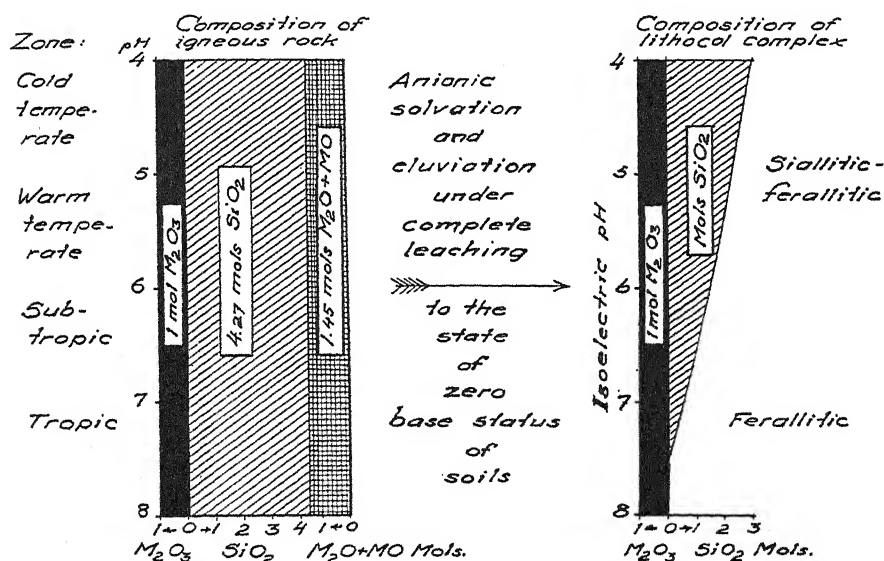


FIG. 115. A SCHEME TO SHOW THE RELATIONSHIP BETWEEN THE COMPOSITION OF THE LITHOCOLS (MINERAL COLLOIDS) AND THE PREVAILING pH AS BASED ON THE COMPOSITION OF THE ISOELECTRICALLY PRECIPITATED SILICATES

more silica will be left in combination with the sesquioxides at the time the soil has reached the state of zero base status. Such soils might be said to be of the siallitic-ferallitic type (cf. temperate humid branch in figure 114).

At the point of zero base status the pH of the soil is very close to that of the I.E.P. (= isoelectric point). The latter has been adjusted by the anionic solvation and eluviation to coincide with the prevailing pH. The pH of the unsaturated ferallitic soils and their I.E.P.'s are, therefore, both relatively high (6 to 7 or more), whereas the corresponding values in the siallitic-ferallitic soils are as much as 2 to 3 pH units lower. This is all the result of an isoelectric weathering by anionic solvation and eluviation.

This process is schematically illustrated in figure 115. The composition



of the original igneous rock is based on the data presented by Clarke (4), which give 4.27 mols of silicate silica (quartz omitted) and 1.45 mols of strong bases to each mol of  $M_2O_3$ . The composition of the completely leached gel is assumed to be the same as the composition of isoelectrically precipitated aluminum and ferric silicates. This composition of the two series of silicates has here been roughly interpolated. The pH values in the different regions refer to the zone of weathering in the soil. In the presence of humus acidoids the pH value may sink considerably below 4, but then the I.E.P. will also be lower. But even in the presence of much humus acidoids, a pH of 4 is probably about the lowest limit for a dominantly anionic solvation of the lithocol complex.

During the process of weathering in the acidic medium the humus acidoids, being stronger than the silica, will cause a partial displacement of the latter. These humus acidoids are not included in figure 115, which deals with the lithocol complex only. The amount of combined silica at the point of zero base status should therefore be smaller than that calculated on the basis of the composition of the isoelectric silicates.

It appears to be chiefly the iron which thus combines with the humus acidoids. Now since the humus is subject to decomposition, ferric oxides will be liberated, and this is, in the author's opinion, the explanation why all unsaturated, siallitic, automorphous soils develop a brown coloration. Such soils should therefore be classified as siallitic-ferallitic. Such soils yield, if sufficiently unsaturated and after the humus acidoids are destroyed by heating (8), an exchange alkalinity in a dilute sodium sulfate solution. The ferallitic soils, of course, show this reaction to an even greater degree.

The theory here outlined is not only supported by the composition of the pedocols (= soil colloids, including lithocols and biocols) in different humid latitudes but also by their complement, the dissolved materials in river water. Thus from the data of Clarke it was found that the ratio silica/strong bases increased progressively from 0.19 to 1.55 from the rivers of the St. Lawrence basin to the rivers of British Guiana (17). This means that the bases carry a greater equivalence of silica in solution in the tropics than in the colder regions, exactly as shown in figure 115.

Silicic acid is not a complete acidoid in the sense of the higher humus acidoids. In the free state, silica is rather soluble. It occupies a position between the humus and the phosphoric acidoids (the amphoteric phosphates which form a completely soluble acid when hydrolyzed). The isoelectric silicates of Al and Fe precipitate in the presence of considerable dissolved silica. The humates are completely precipitated. When silica is liberated from the sesquioxides by the alkaline hydrolysis, it carries little or no Al and Fe in the anionic solvate. It virtually alone is eluviated, therefore. In the solonetz and the solodi, where there is little or no eluviation, the silica is thrown out in the amorphous form.

The humus acidoids behave differently. They form very stable amphoteric compounds with Al and especially with ferric iron. These compounds hydrolyze only partially above or below their I.E.P., at the reactions prevailing

in the soil. The anionic humic solvates will therefore carry considerable Al and especially Fe in "solution" just as the cationic solvates will carry humus acidoids in "solution." The only difference between the two types of solvates, besides the sign of charge, is that the anionic sol complex is richer in humus acidoids and the cationic sol complex is richer in sesquioxides than the parent gel complex (3).

The humus acidoids are always more or less combined with the sesquioxides in the unsaturated, siallitic-ferallitic, and especially in the ferallitic soils. Some of the sesquioxides undergo, therefore, an anionic solvation and eluviation in association with the humus acidoids.

The tendency for this form of eluviation will depend on the nature of the humus acidoids. Humus acidoids formed under the conditions of a high base status possess a much higher capacity to bind base than the humus acidoids formed in an acid medium; in fact, the capacity of the former may be as much as three times that of the latter. The equivalent weight of the former is lower, their molecular aggregates are smaller (= shorter chains?), and their tendency to disperse is greater. The humus acidoids of the "mild" humus are also stronger than the "sour" humus acidoids (23).

In the degradation of the chernozem the humus acidoids are, therefore, more rapidly anionically solvated than in soils formed under more acid conditions. Some Al and especially Fe will be mobilized in association with humus. This may give rise to the appearance of "podzolization." The same explanation accounts for the "podzolization" of the solodi at a pH which precludes any cationic solvation.

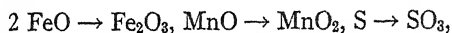
Where the movement of the anionic solvate is upward, as in the laterites during the dry period, a peculiar situation will arise. In the laterites the humus acidoids are formed at a relatively high pH. The humus acidoids should, therefore, possess the aforementioned properties of "mild" humus. Most or all of these acidoids should, therefore, exist in combination with the large amounts of free sesquioxides. The I.E.P. of the latter is thereby lowered, and small amounts of the humates will, at the prevailing pH, undergo an anionic solvation and be carried to the surface by the upward movement of the water. Here the complex would again be eluviated downward during the wet season if it were not for the inevitable fact that the humus acidoids will suffer a decomposition. The sesquioxides are, therefore, irreversibly precipitated and thus form the lateritic crust.

The ferric humates having the lowest I.E.P. will be the most strongly electronegative, will be the first to move and the last to be precipitated. Vageler (28) asserts that the uppermost layer of the crust, as a rule, is enriched in Fe, whereas a little deeper, Al shows the greatest accumulation. This is the opposite to the cationic eluviation in the podzol. Here the Al-humates are most strongly electropositive, are the first to move and the last to precipitate (cf. below). The facts seem, therefore, to confirm the theory that the lateritic crust is the result of an anionic solvation and eluviation.

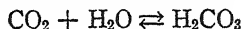
*Cationic solvation and eluviation.* At the zero base status the soil contains

small but equivalent quantities of combined acids and bases. The solvation here is at a minimum. Anionic and cationic fragments may split off, but above a sufficient concentration they will mutually precipitate each other. Figure 116, which is adapted from part XIX of this series (18), shows such a minimum. The soil material is from a podzol B-horizon and gives a minimum at pH 4.7. The pH was adjusted by NaOH and HCl in a NaCl solution.

In the absence of organic and mineral acids the zero base status would be the end state of a leached soil. The laterites are perhaps the only soils which attain this condition (28). Carbonic acid cannot alone bring about a cationic solvation. But oxidation spells decrease in basicity or increase in acidity and wherever oxidizable material gets into a well-drained soil there will be a decrease in bases or an increase in acids:



The only thing preventing nature from running amuck in the next step in the oxidation of organic matter ( $\text{RCO}_2\text{H} \rightarrow x\text{CO}_2$ ) is the fact that the reaction which would form large quantities of a strong acid ( $k_a$  about  $1 \times 10^{-3}$ )



does not proceed very far. Besides, nature had other functions for  $\text{CO}_2$  to perform and so made it a gas.

An accumulation of organic matter must, under leaching conditions, lead to an excess of acids. Having all its acidoid groups occupied by H ions, the basoid groups of the gel complex will begin to function. This will lead to an acid hydrolysis and to a cationic solvation and eluviation.

The pH at which this solvation will begin depends not only on the acidoid/basoid ratio but also on the buffer capacity for acids and on the nature of the acid anions. The latter determine the magnitude of the charge and, within certain limits, the position of the I.E.P. A ferallitic soil will begin to bind acid anions at a relatively high pH, depending upon the nature of the ions, but the cationic solvation does not seem to occur before the pH has sunk to about 5 (28). Siallitic soils and soils having a high content of humus acidoids seem to be stable down to a pH of about 4.

Aluminum compounds solvate cationically at a higher pH than those of ferric iron. This is because alumina is a stronger base and its compounds have a higher I.E.P. than the corresponding ferric compounds. (If a ferallitic soil is treated with ferric chloride, ferric oxide will be deposited and Al will pass into solution.)

The movement of iron in the soil is, however, complicated by its conversion into the ferrous form. All laboratory prepared cationic solvates from podzol profiles have shown the ferrous reaction. Ferrous hydroxide is the strongest base of the three and was found to be isoelectric at about pH 9. In spite of this the ferrous silicates possess a relatively low I.E.P. and are rather soluble even at this point, as is shown in table 161.

The I.E.P. was determined as in the isoelectric precipitation of the aluminum and ferric silicates (15). The precipitates, which were very voluminous around the I.E.P., were dark green at high pH, brownish green at the I.E.P., and at a pH near 5 they became rusty brown and small in volume. The ferrous iron in combination with humus (3), passes into solution at a much higher pH than Al and ferric iron. The oxidation, as indicated by the brown color, took place in spite of all possible precautions. At a pH below 7 all precipitates became de-

TABLE 161

*The composition of isoelectric mixtures of  $\text{FeCl}_2 + \text{Na}_2\text{SiO}_3 + \text{HCl}$  or  $\text{NaOH}$   
Millimols in 2 liters*

NUMBER	IN SYSTEM		IN SOLUTION		IN FLOC		COMPOSITION OF FLOC $\text{SiO}_2/\text{FeO}$	I.E.P.
	$\text{FeCl}_2$	$\text{Na}_2\text{SiO}_3$	$\text{FeO}$	$\text{SiO}_2$	$\text{FeO}$	$\text{SiO}_2$		
I	10.0	3.63	0.63	0.22	9.37	3.41	0.364	pH 6.3
II	10.0	7.25	2.75	2.46	7.25	4.79	0.66	5.4
III	10.0	14.50	4.50	8.29	5.50	6.21	1.13	5.2

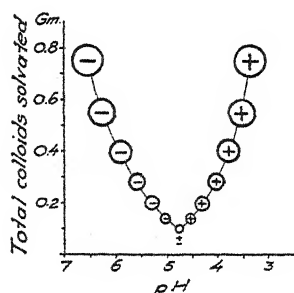


FIG. 116

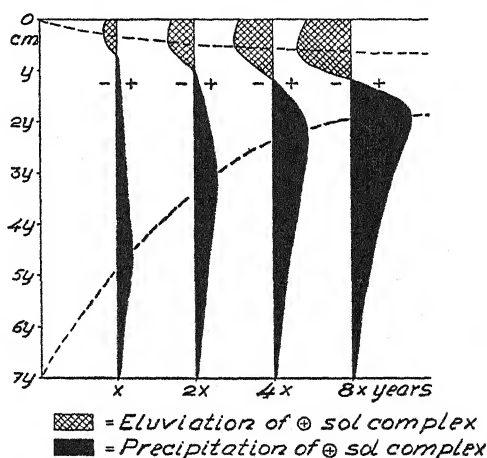


FIG. 117

FIG. 116. A SCHEMATIC REPRESENTATION OF THE ANIONIC AND CATIONIC SOLVATION IN A SUSPENSION OF A PODZOL B<sub>2</sub>-SAMPLE, 200 GM. IN 2 LITERS, THE pH BEING ADJUSTED WITH NaOH AND HCl IN 0.01 N NaCl SOLUTION

The colloids include humus,  $\text{SiO}_2$ , and  $\text{M}_2\text{O}_3$

FIG. 117. THE DEVELOPMENT OF THE PODZOL PROFILE ACCORDING TO AALTONEN  
The figure, modified by the author, shows how the B horizon grows from the bottom up

cidedly more acid (by 2 pH units or more), and the electronegative ones changed to positive by the addition of hydrogen peroxide. These observations give us an appreciation of the complications in the mobility of iron which arise when ferric iron is reduced to the ferrous condition. In the presence of easily oxidizable organic matter some iron is bound to be reduced. The movement of iron in the form of complex anions has been suggested, but this would not account for the precipitation in the B horizon.

The cationic sol complex, unlike the anionic, must always precipitate in the B horizon when it encounters a sufficiently high pH. The lower the I.E.P. of the complex, the sooner it will encounter that barrier below which it cannot pass. Hence the material precipitating in the upper B horizon is richest in humus and is, at least in the drier profiles, relatively richer in ferric iron than in aluminum. In podzolization the order in which the two elements move is, therefore, the reverse of that in laterization, exactly as the theory demands: in podzolization the cationic Al-complex is the stronger positive and moves deeper down, whereas in laterization the anionic ferric complex is the stronger negative and moves higher up.

Aaltonen (1) has made the interesting observation that the younger the podzol profile, the deeper is the illuviation maximum in the B horizon: the horizon grows from the bottom up. A theoretical consideration would lead to the same conclusion because in the early stages of the cationic solvation in the A horizon the parent gel is richer in sesquioxides and will, therefore, at a given pH yield a sol complex with a higher I.E.P. than will be the case later at a more advanced state of eluviation.

Figure 117 is adapted from the work of Aaltonen in a form which has been somewhat modified by Mattson and Lönnemark (21).

*The cationic eluviation in the A horizon and the precipitation in the B horizon lead to a differentiation of the material in such a way that an isoelectric gradient is established which approximates the prevailing pH gradient of the profile. The isoelectric weathering leads to a stabilization of the soil material whether it be by an anionic or a cationic solvation and eluviation or by precipitation.*

"Podzol" means ashlike (from the usually ash-gray color of the A<sub>2</sub> horizon), but it must be clearly understood that the differentiation in the podzol profile must be governed by the acidoid/basoid activity ratio. A podzolized horizon might be bleached, dirty gray or black depending upon the color of the dominant acidoids, i.e., silica or humus.

The exchange reactions (pH in water minus pH in 0.01N Na<sub>2</sub>SO<sub>4</sub>) give the simplest and surest indication of the degree of podzolization (22).

Since the podzols are cationically eluviated only in the A horizon they give rise to what might be called A-siallitic/B-ferallitic soils (cf. fig. 114).

#### *Reaction and base status*

The pH of a soil does not tell us anything about its base status except that, by definition, the soil is 100 per cent base saturated when, in the absence of salts, the pH is 7. A solonchak has a lower pH than a solonetz although its base status is higher. If the salts are leached out of a solonchak it becomes more alkaline in reaction because of a release of bases. A laterite of zero base status has a much higher pH than a gray forest soil of the same base status. The I.E.P. of the laterite is correspondingly higher and it might, for this reason, have a pH as high as an 80 to 90 per cent saturated prairie soil. The belief that the laterites are very acid soils because of their low base content is errone-

ous. As Joffe (10) rightly observes, the base content cannot serve as a criterion.

### *The exchangeable ions*

The division of soils on the basis of the dominant exchangeable cations as proposed by Gedroiz (6) is useful and should be retained.

The displacement of the ions takes place according to the mass law as expressed by the equations

$$\frac{(H^+)_i}{(H^+)_o} = \frac{(Na^+)_i}{(Na^+)_o} = \frac{\sqrt{(Ca^{++})_i}}{\sqrt{(Ca^{++})_o}} \quad (A)$$

and

$$\frac{(OH^-)_i}{(OH^-)_o} = \frac{(A^-)_i}{(A^-)_o} = \frac{\sqrt{(A^-)_i}}{\sqrt{(A^-)_o}} \quad (B)$$

where ( ) denote activity in the inside, *i*, and in the outside, *o*, solution respectively and  $A^-$  and  $A^{--}$  monovalent and divalent anions (22).

From these equations we can make the following deductions:

In high concentrations in the outside solution (approaching that of the inside micellar solution) the monovalent ions have about the same displacing power as the divalent ions. In saline soils, in which the activity of Ca ions is limited to the solubility of the carbonate and sulfate, the Na ions will, therefore, dominate in the exchange complex.

In low concentrations in the soil solution the displacing power of the divalent ions is enormously greater than that of the monovalent ions (0.000001  $Ca^{++}$  would balance 0.001  $Na^+$  in the soil solution at an inside activity = 1). When a soil is leached, therefore, the Ca-ion concentration maintained by the carbonate and sulfate will displace most of the Na ions.

The lower the acidoid content, i.e., the capacity to bind base, the greater will be the dominance of the Ca ions because a larger percentage of the Na ions must be displaced to satisfy the equilibrium. Hence the monovalent ions will be more easily displaced in a sandy soil than in a clay soil or, putting it the other way, a much lower Ca-ion concentration will be required in the soil solution to maintain a given percentage Ca-saturation. A soil of low exchange capacity might be looked upon as a diluted soil of high capacity. If a soil saturated with Ca and Na ions and in equilibrium with a solution of these ions be diluted 100 times with water, then a certain amount of Na will be displaced by Ca. To maintain the original percentage saturation with Ca and Na the Ca ions would have to be diluted 10,000 times, or 100 times more than the Na ions (22, table 115).<sup>1</sup>

<sup>1</sup> In a recent paper Jenny and Ayers (9) have come to the conclusion that the percentage of an ion (K) displaced by another ion decreases with decreasing percentage saturation with K ions. This is contrary to the mass law, according to which the same *percentage* of a monovalent ion should be displaced at a given activity of the displacing ion independently of the degree of saturation. The electrostatic forces must be the same whether a positive charge sits on one or the other atom of the same size, in which case the activity coefficient will be the same. Jenny and Ayers varied not only the percentage saturation but also the amount of soil by adding, for example,  $NH_4$ -clay to the K-clay. For a certain percentage displacement of K under such conditions the mass law demands 100 times greater concentration of the displacing ion in a system containing 1 gm. K-clay and 99 gm.  $NH_4$ -clay than in a system containing only 1 gm. K-clay (assuming the activity coefficients of  $K^+$  and  $NH_4^+$  to be equal). By substituting the activity for the "oscillation volume" and applying the mass law it would seem that the results of Jenny and Ayers would confirm this law but not their conclusion.

The H ions are slightly dissociated by the acidoids. Their activity in the micellar solution being low, they will, even in a low concentration in the soil solution, actively begin to displace the Ca ions.

Before the soil complex has become unsaturated with metal cations it will begin to bind acid anions, the trivalent and divalent ones long before the monovalent. This will tend to conserve the remaining cations.

The pH at which the soil binds an equal amount of anions and cations, i.e., the equi-ionic point (E.I.P.), and the amount combined will, of course, depend on the nature of the ions and on the acidoid/basoid activity ratio in the complex. The lower this ratio and the higher the valence of the anions in relation to the cations, the higher is the E.I.P. and *vice versa*. The more balanced the activity of the acidoids and basoids, that is, the nearer the ratio approaches unity, the greater will be the amount combined. Further, the greater the concentration of the colloid and of the ions in solution, the greater also is, of course, the amount combined. Considerable quantities of ions may thus be held at zero base status, i.e., at the E.I.P. (22).

It should be pointed out that we have no knowledge about the actual quantities of H and OH ions present in the complex. At the E.I.P. a certain proportion of the acidoid and basoid residues must combine to form an inner salt and water. Conversely, at high and at low pH the complex must be hydrolyzed, thus increasing the basoid and acidoid residues respectively. The "excess" Na ions in a supersaturated soil may, therefore, never have displaced any H ions.

It should also be pointed out that the humus layer of acid soils is probably very seldom negatively saturated with bases, except under special conditions, as when sulfides are oxidized to sulfuric acid in large amounts. The humus acidoids are themselves stronger than most weak acids and can not, therefore, be fully desaturated by the latter. The mineral acids are usually more than compensated by the strong bases.

#### THE MORPHOLOGY OF THE PEDOSPHERE—SOIL TYPE AND SOIL SERIES

The anatomical parts of a body are differentiated on the basis of their morphology. The soils within a group will differ in an analogous way to the tissues of an organ. The morphological differences of soils give us the soil type, and this must therefore be based on soil profile characteristics. These should include the nature of the vegetation, the "V-horizon" which properly belongs to the soil profile, in addition to the profile characteristics usually studied.

Soils show all gradations in composition and properties. A limited number of type names, therefore, can not adequately classify all soils. The merit of the system proposed here (fig. 114), if it has any, is that it obviates this limitation by making a graded classification possible. Wherever a main type is established it will be found to grade continuously with respect to its humus content and base status into the adjoining main type. Each main type forms,

therefore, whole series in which the differences can be expressed by numerical values with respect to humus content and base status and thus be located on the chart.

In the dominantly automorphous (climatogenic) soils the series will be zonal and might, therefore, be called "geoseries." The dominantly hydromorphous (hydrogenic) soils are azonal and form hydrologic series.

The geoseries must necessarily show an orderly arrangement when charted as in figure 114. Thus, from the neutral chernozem series, high in humus, to the supersaturated serozem series, low in humus, the geoseries must occupy a certain belt on the chart (the tropical arid zone). Whether the "curve" along which the names are written represents, even approximately, the true relationship, the writer does not know. It is merely intended to show the trend. The northern chernozems in the United States would undoubtedly occupy a different position from that of the southern, for example.

The leached, unsaturated, and negatively saturated geoseries are placed in three zones which branch off from the degraded chernozems and the prairie soils, to wit, the temperate humid zone, the tropical humid, and the tropical-humid/arid zone. Here again the position of the "curves" with respect to the humus content of the main type of each series is subject to adjustment. With respect to the humus content the series of the different zones must be expected to overlap.

The chernozems are assumed to occupy the peak with respect to the humus content, which in humid zones decreases with decreasing base saturation, the more so the higher the temperature. The laterites may become slightly negatively base saturated but they never become podzolized, since no plants can grow on their fully developed crust (24).

The dominantly hydrogenic (hydromorphous) soils occur as azonal types wherever the ground-water level is high enough to dominate the soil processes. They form locally, often within a few meters (21), hydrologic series from one to another type of soil. Any of the zonal soil types must grade into a hydrologic series wherever an impeded drainage so dictates. Thus the zonal iron podzol will develop into an iron-humus podzol, a humus podzol, and a bog soil (27). The more or less base-saturated zonal soils will grade into meadow and marsh soils or other allied soils. This is indicated by arrows in the figure. The hydrologic series will, of course, often show a bilateral gradation, with the drier forms (less humus) on one side and the wetter forms on the other side of the main type. A hydrologic series, further, may be more or less complete or it may be fragmentary.

The completely subhydric soils like the bog soils are, with some exceptions (12) not cationically eluviated. The pH of the ground water is usually relatively high, and the soils, therefore, are more or less base-saturated. Ferrous iron is removed in solution, but both ferrous and ferric iron and aluminum might to some extent be anionically solvated and eluviated with the humus.



## TEXTURAL VARIETIES

We have compared the soil types, as parts of the pedosphere, to the tissues of an organism. Now, tissues are made up of histological units, and soils are in an analogous way made up of textural units. On the basis of their texture, soils are classified into textural varieties according to the generally adopted methods of mechanical analysis.

## THE PEDOCOLS

The histological units of an organism, the cells, contain the ultimate units of living matter in the form of the colloidal protoplasm. Even here our analogy is applicable. The ultimate, aggregate units of the pedosphere, which we collectively call the "pedocols," might be compared to the protoplasm of the living organism.

The pedocols consist of lithocols and biocols.

The biocols represent a continuous series of ampholytoids from those having a relatively high I.E.P., a low acidoid strength, and a low base-binding capacity, i.e., "sour" humus, to those having a very low I.E.P. with strong acidoid properties and a high base-binding capacity, i.e., "mild" humus (23).

The lithocols are of at least three kinds:

Primary silicate particles of colloidal dimensions such as those found in the glacial clays ("rock flour")

Secondary clay minerals such as the kaolinite, montmorillonite, and illite series. (Cf. a great number of publications on X-ray analysis by Endell, Grim, Gruner, Hofmann, Hendricks and Fry, Kelley and coworkers, and Marshall and others.)

The heterogeneous gel and sol complex of indefinite composition consisting primarily of combinations of the hydrous oxides of Al, Fe, and Si and compounds of C (humus). For this complex the name "alfesic" ( $\text{AlFeSiC}$ ) complex has been proposed (19).

The alfesic complex exists in the soil as discrete aggregates and as surface layers on the crystalline particles. Each mineral particle possesses a micro-pedosphere of its own, a layer of a different composition from that of the interior, resulting from the interaction of the free surface valencies with the material of the external medium. The alfesic complex assumes a higher I.E.P. the farther the anionic solvation and eluviation have progressed. The unsaturated siallitic-ferallitic and, especially, the ferallitic soils yield, therefore, an exchange alkalinity in a dilute sodium sulfate solution if the humus, if abundant, is first destroyed by heating (to about 275°C.).

To what extent the clay minerals possess amphoteric properties has not been determined. Electrodialyzed siallitic soils yield no exchange alkalinity in neutral salt solutions. Remozow (24) found that among the anions only the phosphate ion was adsorbed by such soils.

The colloidal behavior of glacial clays (primary silicates) is much less pronounced than that of chemically weathered clays (secondary, hydrous clay-minerals) as measured by the volume of floc and by the swelling. Thus an ultrafraction of the glacial Ancyclus clay (exchange capacity = 31 m.e./100 gm.)

swelled, in the Na-saturated condition, to a volume of only 1.6 cc. and yielded a volume of floc of only 20 cc. per gram. The ultrafraction from a completely decomposed amphibolite bed (exchange capacity 84 m.e./100 gm.) gave a swelling of 10.7 cc. and a volume of floc of 158 cc. per gram, which is of the same order as that of bentonite. This difference is probably due to a difference in the number of particles per gram. The reported lattice swelling could hardly account for the differences.

The colloidal behavior and the capacity to bind base of the secondary lithocols vary greatly with the position of the I.E.P. and therefore, in a general way, with their composition. The siallitic colloids having a low I.E.P. possess a high capacity to bind base, swell greatly in the Na-saturated condition, yield a voluminous floc, and are highly plastic. The ferallitic colloids possess these properties to a much smaller degree (16). The particles appear to be smaller in the former than in the latter type (2). The writer has attempted to explain this on the basis of the theory that there must be a certain relationship between the number of charges and the surface, that is, the particle size would attain stability only at a certain density of charge (14).

Among the most important colloidal factors in soil classification the following might be given:

The amphoteric behavior as expressed by the exchange reaction of the electrolyzed soil or colloid in a dilute sodium sulfate solution.

The "colloidal" as expressed by the volume of floc of the clay fraction obtained in the mechanical analysis.

The composition of the colloidal fraction with reference to the silica/sesquioxide ratios. The base-exchange capacity.

Of these the first two factors are very easily evaluated and should, therefore, prove useful.

#### THE SOIL SOLUTION

The soil solution exists in equilibrium with the amphoteric pedocols and is subjected to constant variation, with respect to both composition and concentration. The physiological condition of the soil depends on the concentration of the essential ions in the soil solution and on the potential capacity of the adsorbing complex to supply these ions. The most important physiological factors of the soil are the pH and the KPN status.

The H-ion concentration in the soil solution is governed, among other things, by:

The I.E.P. of the amphoteric complex.

The degree of saturation with acid or base.

The concentration and nature of the other ions (22).

The exchangeable K ions in the soil are distributed between the soil complex and the soil solution according to the mass law (cf. equation A).

The relationship of the phosphate ions to the soil complex is much more

complicated. These ions enter into a "colloid" (as distinguished from "saloid") binding with the soil basoids. The stability of these compounds is greatest at the I.E.P. (20). The phosphate ions are released by hydrolysis above and below the I.E.P. This hydrolysis is suppressed by the presence of soluble salts and so is the phosphate "solubility" (11). At high pH the phosphate solubility is reduced by an extramolecular precipitation with other ions (5).

Concerning the nitrate ion it should be pointed out that this anion, like all other anions, combines, in a saloid form, with the basoid groups of the complex. In ferallitic soils this adsorption must be appreciable.

#### SUMMARY

Soils should be looked upon as parts or "tissues" of a larger body, the pedosphere. By a biological analogy the following division is made:

The body = the pedosphere.

The anatomy = the great soil groups.

The morphology = the soil type as based on profile characteristics.

The histology = the textural units.

The protoplasm = the colloidal complex.

The physiology = the soil solution.

The classification is based on the following factors:

Groups (nine): Humus content and base status.

Types and series: Profile characteristics.

Varieties: Texture.

Colloid-chemical factors: Composition, amphoteric and colloidal behavior of colloids.

Physiological factors: pH and KPN status.

The soil-forming processes in relation to anionic and cationic solvation and eluviation have been discussed.

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## BOOKS

*The Leguminous Plants of Wisconsin.* By NORMAN C. FASSETT, RICHARD I. EVANS, AND CATHERINE MOSE. The University of Wisconsin Press, Madison, Wisconsin, 1939. Pp. 157, illus. 97. Price, \$3.

The purpose of this book is to record the taxonomy, ecology, and distribution of the Leguminosae growing in Wisconsin without cultivation. Keys to the several species are based on vegetative characters, flowers, fruits, and seeds. The illustrations are particularly interesting and show the several parts of the plants in concise detail.

*Modern Fruit Production.* By JOSEPH H. GOURLEY AND FREEMAN S. HOWLETT. The Macmillan Company, New York, 1941. Pp. 579, illus. 87. Price, \$4.50.

Modernized methods of soil and plant management, as applied, primarily, to the production of tree fruits. Almost any question which one might ask on this subject, whether about the design or the function of any part of the tree, is discussed and illustrated. The craftsmanship of this book is especially noteworthy.

*My Country 'Tis of Thee.* By LUCY SPRAGUE MITCHELL, ELEANOR BOWMAN, AND MARY PHELPS. The Macmillan Company, New York, 1940. Pp. 335, illus. 91. Price, \$3.50.

Designed to add feeling to thought with reference to the use and abuse of three natural resources—soil, coal, and oil. The book is written for anyone not “too young to think and not too old to feel.” Numerous attractive illustrations add interest to the presentation and widen the scope of usefulness of the volume.

*Photosynthesis.* By E. C. C. BALY. D. Van Nostrand Company, Inc., New York, 1940. Pp. 248. Price, \$4.75.

This is a report of investigations of the process of photosynthesis, the final result of which was the laboratory production of carbohydrate from carbon dioxide and water. Additional chapters deal with the kinetics and mechanism of photosynthesis and with the assimilation of nitrogen by living plants. A very stimulating discussion of a very interesting subject.

*Soils and Soil Management.* By A. F. GUSTAFSON. McGraw-Hill Book Company, Inc., New York, 1941. Pp. 424, illus. 165. Price, \$4.

This book is designed primarily for teaching purposes, but will be found useful by practical farmers as well as college students. It contains 19 chapters

dealing with such subjects as organic matter, soil moisture, cultivation, erosion, acid and alkali soils, crop rotation, green manures, animal manures, and fertilizers. The last chapter has to do with peat soils and their management. A list of practical questions is appended to each chapter.

*Temperature, Its Measurement and Control in Science and Industry.* Edited by a Publication Committee of the American Institute of Physics. Reinhold Publishing Corporation, New York, 1941. Pp. 1362. Price, \$11.

This large volume is a record of a "Symposium on Temperature" which was held in New York City, November 2, 3, and 4, 1939, under the auspices of The American Institute of Physics. Almost every phase of temperature measurement and control is covered in this report by men who are exceptionally well qualified in their respective fields. The several papers are classified under such headings as temperature scales, precision thermometry, education, natural sciences, biology, man, regulation and recording, special applications, general engineering, metals, ceramics, oil, and optical and radiation pyrometry. Anyone dealing with any phase of temperature research or control will find this an extremely useful book for ready reference.

*The Theory of Ground-Water Motion.* By M. HUBERT KING. Comprises the entire Number 8 issue of the Journal of Geology. University of Chicago Press, 1941. Pp. 785-944, illus. 48. Price, \$1.25.

A reexamination of the fundamental principles of ground-water motion designed to clarify some previous misconceptions and to establish the subject on a fundamental basis in conformity with the laws of thermodynamics. Of particular interest to soil physicists.

THE EDITORS

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#### GOVERNMENT WANTS METEOROLOGISTS

SOIL SCIENCE has been requested by the United States Civil Service Commission, in furtherance of the current national policy, to bring to the attention of its readers an announcement of a civil service examination to obtain meteorologists for the Federal Government, at salaries of \$2,600 to \$5,600 a year, less the usual retirement deduction. Applicants are especially desired who have had experience in meteorological research or in practical work in forecasting, according to the Commission. Separate employment lists will be set up in such specialized branches of meteorology as climatology, dynamic meteorology, and radiometeorography, the announcement points out.

Although applications will be rated as received at the Commission's Washington office until December 31, 1941, the notice continues, qualified persons are urged to apply at once. Further information and application forms may be obtained at any first- or second-class post office, or from the Civil Service Commission, Washington, D. C.

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## PRESENT CONCEPTS OF ION AVAILABILITY IN PLANT NUTRITION

This number of *SOIL SCIENCE* is devoted exclusively to five papers that were presented before a joint session of the American Society of Plant Physiologists, the American Society of Horticultural Science, and the Physiological Section of the Botanical Society of America at the Philadelphia meetings of the American Association for the Advancement of Science on Tuesday afternoon, December 31, 1940. The symposium was held in the Auditorium of the University of Pennsylvania Museum, and was attended by some 500 plant and soil scientists, under the chairmanship of Dr. Richard Bradfield, Head of the Department of Agronomy, Cornell University, at whose suggestion this collective publication of the papers was arranged for. We are glad to have the opportunity to present this relatively complete picture of the present knowledge on this important subject. Of great importance alike to both plant and soil scientists, nutritional problems present a common meeting ground for these diverse interests.

FIRMAN E. BEAR





## PHYSIOLOGICAL ASPECTS OF AVAILABILITY OF NUTRIENTS FOR PLANT GROWTH<sup>1, 2</sup>

D. R. HOAGLAND AND D. I. ARNON

*University of California*

Received for publication March 4, 1941

The term "availability" as it is used with respect to inorganic plant nutrients is seldom defined with precision. It may imply some empirically determined chemical property of a soil or the interreaction of many chemical, physical, and physiological factors. Some investigators have suggested modifying terms to express different aspects of availability of nutrients, especially of potassium and phosphate, such as chemical availability, physiological availability, physical availability, "weathering" availability, and "positional" availability. In some of these concepts the idea of accessibility is involved, as are also time factors. Because of these complex meanings that may be attached to the term, we shall not speak very much in this paper of availability in general, but shall discuss primarily specific phases of the system of plant and nutrient medium with reference to the absorption of inorganic solutes by the plant.

### NATURE OF PHYSIOLOGICAL PROCESSES INVOLVED IN ION ABSORPTION

At the outset it seems desirable to restate several problems concerning the general nature of the processes of ion absorption and accumulation by plants (10). There are several subdivisions of the subject: (a) the first step in the withdrawal of ions from soil solutions or artificial nutrient solutions, or perhaps directly from the surface of soil colloids; (b) accumulation of ions in root protoplasm or vacuoles and concomitant or subsequent movement of ions into the conducting system of the plant; (c) upward movement and distribution of ions throughout the plant.

Let us consider the simplest case, represented by experiments on excised roots immersed in and absorbing ions from a dilute salt solution. For rapid absorption, healthy and metabolically active roots are required which have not already absorbed and retained salt in amounts approaching an equilibrium condition. Far greater quantities of ions will then be capable of absorption

<sup>1</sup> It is not possible to present a general review of literature in this paper. Most of the experiments cited by way of illustration were carried out by various members of the California Agricultural Experiment Station.

<sup>2</sup> EDITOR'S NOTE: The eighteenth annual award by the American Association for the Advancement of Science of \$1,000 for a notable contribution to science was bestowed on the authors of this paper, which was selected from among the papers presented at the meetings of the association in Philadelphia, December 27, 1940 to January 2, 1941.

by the roots under aerobic than under anaerobic conditions. In fact, only when given an adequate supply of oxygen do the roots show ability to accumulate ions; that is, to move them from a dilute external solution to a more concentrated internal solution, an uphill process that demands the expenditure of cellular energy. The temperature coefficients of this process of ion accumulation are high, within a biological range. The essential protoplasmic activities of roots which result in ion accumulation are reflected in oxygen absorption or in the aerobic production of  $\text{CO}_2$ , but are not stoichiometrically related to respiration. This oxidative metabolism requires a store of available carbohydrate and presumably certain growth substances. Therefore the temperature and the oxygen supply of the nutrient medium and indirectly the photosynthetic functions of the shoot are among the indispensable factors determining rates of active absorption of ions [cf. reviews (11, 15)].

From comparisons of tomato plants grown to stages of heavy fruiting in water, sand, and soil media in large-scale greenhouse cultures (5), we have had indications of the practical importance of a liberal oxygen supply as a determinant of nutrient absorption and of other physiological processes. The sand culture and the water culture, provided forced aeration was employed in the latter case, gave considerably higher yields of fruit and greater absorption of nutrients than a well-fertilized soil in good physical condition—a soil which had a history of excellent production of tomatoes in a commercial greenhouse and which gave an unusually high yield under our conditions. It would seem that in a soil the factors of oxygen supply and of  $\text{CO}_2$  removal from around the roots may sometimes limit nutrient absorption by plants, even when availability of nutrients, in a chemical sense, is not limiting. It is, however, necessary to note that oxygen requirements of roots and the effects of  $\text{CO}_2$  may not be alike in magnitude for different types of plants.<sup>3</sup> There are also instances in which injury has been produced by overaeration of roots. This detrimental effect would be enhanced at higher temperatures conducive to an excessive rate of respiration in the root tissues.

Another consideration regarding absorption of ions by roots of high absorbing capacity is important to the present discussion. Absorption curves, at least for mobile ions like potassium, when plotted against concentration in the nutrient solution, rise very steeply in a low concentration range, for example: 0.0001 to 0.001 *M*. Under conditions favoring active salt absorption, much greater amounts of the ions, relative to concentration, are absorbed from very dilute solutions than from those of higher concentration, within a limited time. Some ions in the culture solution may be rapidly reduced in concentration to exceedingly low values by root absorption, when the volume of solution is limited. To illustrate, in an experiment with young barley plants in which an appropriate relation of volume of nutrient solution to number of plants was arranged, the initial potassium concentration of 3 m. e. per liter in the

<sup>3</sup> There is evidence to suggest that in some plants oxygen may be translocated from the shoot to the root system.

solution was reduced in 24 hours to 0.02 m. e. per liter.<sup>4</sup> (At the same time a high concentration of potassium was present in the root sap.) Thus the efficiency of potassium absorption was very high. Of course, the phenomena are actually more complex than these statements indicate. All the interionic relations in absorption are to be evaluated, as well as the specific characteristics of individual ions influencing rates of entry; for example,  $\text{SO}_4$  ions tend to be absorbed much more slowly than  $\text{NO}_3$  ions. Furthermore, different species of plants growing in the same nutrient solution may absorb the various ions at markedly different rates, and even with plants of the same species the rates vary at different stages of growth and under different environmental conditions.

An interesting aspect of the general problem under discussion is concerned with the relations of transpiration, light, and metabolism to the diurnal course of ion absorption. A water-culture experiment with young barley plants showed that the absorption of nutrients took place about as rapidly in the dark period of a diurnal cycle as in the light period.<sup>5</sup> In this experiment, plants were grown in a greenhouse for several weeks. Some of the plants received nutrients only during the day periods, others only during the night periods, and a third set during the full 24 hours. In summer the total absorption of each of the principal ions present in the solution was nearly twice as great for 24 hours as for either of the 12-hour periods. In winter, however, although the absorption for the 12-hour periods remained approximately equal, the total absorption, when salt was supplied for the full 24 hours, was less than double that for a 12-hour period. This appeared to have been related to reduced illumination as it affected the synthesis of sugar or other metabolites and the resulting limitation in the growth of roots and their absorptive capacity for ions.

There is an upper limit to the amount of an ion that may be held by the roots. To ensure rapid ion intake the roots must not only be metabolically active but must also be maintained well below their maximum salt-holding capacity. The forces which determine the removal of absorbed solutes from the root to the shoot do, therefore, indirectly affect absorption from the nutrient medium. In young plants with sufficiently active root systems, in the absence of transpiration, root pressure is involved in the upward movement of solutes. When larger plants are considered, however, transpiration is probably chiefly responsible for accelerating the removal of salts from the root to the shoot, although there is no necessary proportionality between the amount of water transpired and the amount of solutes absorbed or translocated. A discussion of the problem of movement of ions into the xylem system and the possible role of metabolic activities in this step in translocation exceeds the scope of this paper. We also leave aside the question of solute movement in phloem tissues.

The preceding remarks are based on experiments with plants grown in water

<sup>4</sup> Unpublished experiments of D. R. Hoagland and T. C. Broyer.

<sup>5</sup> Unpublished experiments by D. R. Hoagland and T. C. Broyer.

or sand culture. But there is another approach pertinent to our discussion, which also leads to emphasis on the metabolic activities and growth processes of plants. When a stirred nutrient solution is employed, or a sand culture through which nutrient solution is frequently percolated, the nutrient solution is brought to the roots, whereas in a soil the roots explore the medium in which the nutrients are present. Obviously, then, metabolic activities of roots in soil are of great significance because of their relation to the rate, extent, and kind of root growth. Dittmer (8) estimated that a single rye plant, under very favorable conditions, could develop a root surface (including root hair surface) of about 7000 square feet, with a total root length of more than 350 miles. Clearly, soil and climatic factors directly or indirectly determining root growth must constitute a most important consideration for understanding availability of nutrients in its physiological aspects. We need to know not only the kind of soil, but also the amount of soil accessible to the plant. The implications in terms of soil aeration, soil temperature, compacted soil zones, toxic substances, and other factors affecting the development of roots are obvious.

Before leaving this section of our paper, we wish to note that, though the present treatment demands stress on actively growing and metabolizing roots, it does not follow that absorption of salt can not occur by way of inactive or injured roots, under the influence of transpiration, as a sort of wicklike process. This could be illustrated by an experiment with solutions of increasing sodium salt concentration, including a range in which marked injury to the root system was produced. This question is not without interest, but its further discussion cannot be undertaken here.

Another question should also be raised: What is the relative efficiency of additions of nutrients accessible to a small versus a large part of the whole root system? Here, too, we can do no more than suggest the question, which has, incidentally, a bearing on localized application of some fertilizers to soils, a practice which may be necessary or advisable because of the fixing power of the soil.

So far we have tacitly assumed that absorption of ions proceeds from a nutrient solution in sand or water culture or from a soil solution. During recent years, an additional type of mechanism to explain ion absorption by roots has received much attention, particularly with reference to potassium and other bases. We find it useful to refer here to recent experiments in this laboratory on "contact effects" (13). Evidence has been obtained that cations held on the surface of a soil colloid may undergo exchange with cations present on the root surfaces (including H ions which are, of course, available as a metabolic product of roots) through overlapping oscillation volumes of adsorbed ions. The soil solution, in the ordinary sense, is not a part of the system.

It still remains to learn, however, what the relative importance of the soil solution and the contact mechanisms may be for different ions and under diverse soil conditions. The importance of the soil solution as a direct source of nutrients is manifested by the fact that an ion of major quantitative im-

portance, namely, the nitrate ion, is present primarily in dissolved form and must be accompanied by equivalent amounts of cations. Also, the chloride and sulfate anions, with accompanying cations, are to be taken into account, and bicarbonate ions may often be present in considerable concentration. Frequently, however, soil solution concentrations of potassium and phosphate are very low at any moment, and rapid entry of these ions into the soil solution from solid phases would often be required to explain on a soil solution basis their actual absorption by the plant. But again we are not yet sure that in soils capable of supplying enough potassium to a crop, the low concentration of potassium sometimes occurring in the soil solution is necessarily inadequate. We recall the capacity of active roots for rapid absorption of potassium from extremely dilute solutions and the potentialities for root surface development. Yet it is reasonable to expect that this valuable new point of view on contact absorption will lead to explanations of some soil-plant phenomena which are at present obscure. It is to be observed that our remarks on metabolic factors governing root growth and surface development are also pertinent to discussions of nutrient absorption on the basis of the contact theory.

#### MICRONUTRIENT ELEMENTS

Much of the progress in the understanding of the inherently complex soil-plant interrelations can be traced directly to the development of controlled methods of experimentation. The growing of plants in water and sand cultures<sup>6</sup> has contributed to an appraisal of the function of the soil as a source of plant nutrients. By these means increased understanding has been gained of the essential elements which the soil must supply to the plant for the completion of its life cycle. In addition to the long-recognized indispensability of nitrogen, potassium, phosphorus, calcium, magnesium, sulfur, and iron, positive evidence has been amassed in the past two decades of the essentiality of the four micronutrients: boron, manganese, zinc, and copper. The availability of the micronutrient elements now becomes a definite problem which may also complicate the study of other nutrient elements needed by plants in larger amounts.

It is obvious that only by scrupulous attention to experimental technique, involving rigid purification of the nutrient medium, can the essentiality of elements required in only minute quantities be demonstrated. Yet strong confirmation of the practical importance of these micronutrients is made manifest

<sup>6</sup> As evidence of the applicability of artificial culture methods in the study of plant nutrition, it is worthy of note that the following kinds of plants have been grown successfully in this laboratory in purely inorganic media: barley, rye, wheat, corn, broad bean, kidney bean, alfalfa, field pea, sweet pea, clover, pear, plum, apricot, redwood, pine, squash, cucumber, asparagus, tomato, lettuce, rhubarb, spinach, cosmos, marigold, begonia, cocklebur, mustard, and Bermuda grass. The addition of vitamins or other organic substances was not necessary. Hence for these many types of plants no indispensable factor for plant growth is present in a soil and absent from an artificial medium, however important for other reasons organic matter may be under soil conditions.



by many field observations on deficiency diseases. Of the large number of diverse species of higher plants with which experiments have been made in this laboratory in recent years, none has failed to show typical deficiency symptoms in the absence of each of the four micronutrients, provided suitable safeguards of technique were observed (2, 16). This is in agreement with the results of those earlier workers who appreciated the importance of refined technique in studies of this kind. In the last several years evidence has become available from laboratory experimentation that another micronutrient, molybdenum, is to take a place on the list of essential elements for higher plants (3, 12). Although no proof of direct deficiency of molybdenum has, so far as we are aware, yet been reported for higher plants grown in the field, the importance of this element for the growth of lower plant forms and particularly of its function in nitrogen fixation by *Azotobacter* is of agricultural interest.

The question may be asked whether the list of essential elements as it stands today is complete. Although a number of different species of plants have been grown successfully in this laboratory in a highly purified nutrient solution which by intention comprised only the aforementioned elements, an unequivocal answer to this question cannot be given. It is still possible that, despite all caution, minute impurities were present in the nutrient medium. What can be asserted definitely is that if an element now regarded as dispensable should at some future time be found essential, it will be shown to be required in exceedingly small amounts—within the limits of contamination still encompassed by the refined methods used. A further discussion of this point of view is given elsewhere (2).

#### INTERDEPENDENCE OF FACTORS IN THE STUDY OF AVAILABILITY

A particularly pertinent consideration in discussions of physiological availability is the interrelation between several factors in the environment of the plant. In a study of the relative merits of ammonium and nitrate ions as sources of nitrogen for barley (1), it was found that the assimilation of ammonium, unlike that of nitrate nitrogen, was facilitated by a high oxygen supply or by the availability of a heavy metal like manganese, which by virtue of its chemical properties can act as an oxygen catalyst. The findings suggested that the nitrate as contrasted with the ammonium ion may be of value to the plant not only as a source of nitrogen but also as a source of oxygen. The utilization of either form of nitrogen was influenced by the season of the year. Among the climatic factors fluctuating with season, light, as it affects the synthesis of carbohydrates, which provide the carbon skeleton for organic nitrogen compounds, appears to be of particular significance in the utilization of inorganic nitrogen.

The interdependence of factors in the nutrient medium is also well illustrated by the effects of hydrogen-ion concentration on the growth of plants. High acidity in a soil is frequently associated with unproductivity, but it does

not follow that low pH *per se* is responsible for poor growth. Thus although it is not uncommon for an acid peat soil to support good growth of many kinds of plants, the same cannot be said of a highly acid mineral soil. Indeed, in describing plant responses, the pH of a soil—ignoring for the moment the difficulty of measuring or deciding what the pH of a soil really is—can only have meaning when interpreted not as an independent variable but as a measurement reflecting the interaction of several factors peculiar to a given soil. In a mineral soil, but not necessarily in a peat soil, a low pH is in general symptomatic of calcium and not infrequently of magnesium deficiency, or of the presence in solutions of certain soils of toxic amounts of aluminum or manganese. On the other hand, poor plant growth in a soil characterized by an alkaline pH may in some cases be caused not directly by a high hydroxyl-ion concentration or the presence of alkali salts, but rather by the unavailability of such plant nutrients as phosphate, iron, or manganese.

Experimental verification of this point of view was provided by a recent investigation of the relation of hydrogen-ion concentration to the growth of higher plants under controlled conditions.<sup>7</sup> Tomato, lettuce, rhubarb, and Bermuda grass plants were grown in nutrient solutions maintained within a pH range from 3 to 9. An attempt was made to ensure constancy of composition of the nutrient solution throughout the pH range by reducing the concentration of the nutrient ions to a level low enough to avoid precipitation of phosphates at the most alkaline reaction. To guard against precipitation of iron and manganese above pH 7, these metals were added in the form of synthetic humates. The experimental technique made it possible to interpret the effects of the external pH on the growth of plants in terms of an isolated variable.

At pH 3 complete failure of growth was observed with all the species grown. Some growth occurred at pH 4, and with decreasing acidity, good growth at pH 5, 6, 7, and 8. A decline was noted at pH 9. The results indicated that profoundly adverse effects of hydrogen- or hydroxyl-ion concentration as such are encountered only at extremes of acidity or alkalinity. Within a considerable range, fluctuations in the pH of growth media have not been found to be inimical to the welfare of plants so far studied, provided, of course, that as previously mentioned, such indirect consequences of reaction as toxicity from dissolved soil constituents or deficiency resulting from precipitation of nutrients are avoided. This conclusion is in harmony with the results of an earlier investigation of the influence of hydrogen-ion concentration on the utilization of nitrogen (1).

One possible cause of the injury from extremes of hydrogen- or hydroxyl-ion concentration to plants grown in a nutrient solution is the interference with

<sup>7</sup> Arnon, D. I., and Johnson, C. M. Influence of hydrogen ion concentration on the growth of higher plants under controlled conditions. Paper presented before the Amer. Assoc. Adv. Sci., December, 1940.

absorption of nutrients. The results of the following experiment brought to light a relation between high acidity and calcium absorption.<sup>8</sup> Tomato plants were grown under favorable conditions for about 5 weeks and were then transferred for 96 hours to a series of nutrient solutions maintained at various pH values ranging from 3 to 9. For 3 days prior to the absorption test the plants were kept in a minus-calcium solution. Analyses of the nutrient solution before and after the absorption period disclosed that though the plants absorbed considerable amounts of calcium from cultures maintained at pH 5, 6, 7, 8, and 9, only very little was absorbed at pH 4. At pH 3, however, no calcium was absorbed and the roots were definitely injured.

The relation of calcium to growth at acid reactions was further demonstrated by a series of experiments in which plants were grown in nutrient solutions maintained at pH 3, 4, 5, and 6. Three levels of calcium—20, 80, and 280 p.p.m.—were supplied at each reaction. At pH 6 similarly good growth was obtained at all three levels of calcium, but at pH 4 and 5 the low level of calcium was distinctly unfavorable. A noticeable increase in growth as reflected by fresh and dry weight was obtained at pH 4 and 5 from the higher concentration of calcium in the nutrient medium. But at pH 3, failure of growth was complete at all three calcium levels. It was obvious that for all the plants a reaction of pH 3 was totally unsuited for growth, regardless of the concentration of calcium.

At acid reactions the plant seems to require a greater supply of calcium than at reactions approaching neutrality. This conclusion is of particular interest in the light of the aforementioned relation between high acidity and low calcium supply in mineral soils. Under these circumstances an increased physiological demand for calcium coincides with a decreased supply. It does not seem unreasonable to suppose that the adaptability of "acid-loving" plants to their environment may be in part, at least, due to a low calcium requirement. Also the good plant growth sometimes encountered in acid mucks or other acid soils rich in organic matter may be related to the high calcium-supplying power of these soils. It will likewise be of interest to institute further inquiries on the relation of organic matter to the availability of metals in the form of humates under alkaline soil conditions.

Another consideration which needs to be taken into account in assessing the nutritional adequacy of a soil is the variable physiological demand for certain nutrients at different stages of plant development. The special requirements for phosphorous at the fruiting stage will serve as an illustration of this general subject. In an experiment with radioactive phosphorus, the absorption of phosphorus by developing tomato fruit was found to be very high (4). Under a restricted external phosphorous supply the fruit retained its capacity for phosphorus absorption, but phosphorus was withdrawn from the leaves. To satisfy the needs of both foliage and fruit a large supply of phosphorus was required. Similarly striking evidence of nutrient requirement

<sup>8</sup> See footnote 7.

in relation to fruiting is found in studies on potassium. A particularly favorable set of climatic conditions conducive to great fruitfulness may thus cause an otherwise adequate soil to become deficient as a source of elements required in large amounts in the development of the fruit. It may happen that the full capacity of the plant for absorption of these elements even from a good medium cannot fulfill the physiological requirement. The importance of climatic conditions in interpreting nutritional requirements of plants warrants particular emphasis. The more a given set of climatic conditions favors the development of special organs such as fruit or tubers, the greater is the quantitative requirement of all the inorganic elements which enter into the composition of these plant tissues.

The dependence of nitrogen assimilation on temperature and on light as it affects the carbohydrate reserve in the plant is well known. Other specific relations between climatic factors and mineral requirements of plants have been brought to light in recent years. One example of a micronutrient element may be cited. Corn plants were grown in a greenhouse in a sandy California soil, known for its low zinc-supplying power in the field. In the winter season, although the plants made considerable growth, there was little evidence of zinc deficiency; but in the summer, almost complete failure of growth occurred on this account. Similar seasonal effects were observed with plants grown in nutrient solutions deficient in zinc.

#### THE APPRAISAL OF SOIL DEFICIENCIES

The complexity of the interrelations between the plant and its nutrient medium leaves scarcely a hope for finding a simple solution to this problem. That the problem is in part physiological is illustrated by the different behavior of various species of plants toward certain sources of phosphate. In pot tests with a California soil noted for its high fixing power for phosphate and its incapacity to supply phosphate for most crop plants in the field, tomato plants made scarcely more growth than they would in an artificial nutrient medium free of phosphate, yet buckwheat plants made some development in this soil under similar greenhouse conditions. In a field experiment with this soil, eight species of fruit and nut trees have made good growth and have borne crops which compare favorably both in quantity and quality with those from average trees growing in soils higher in phosphate.<sup>9</sup> The trees in the course of years must absorb a considerable amount of phosphate, although by a test of solubility in a mineral acid the phosphate should be almost completely unavailable in a soil of the kind under consideration. It is not released from the soil colloids by carbonic acid, but rather by OH ions.

What is the explanation of the differentiated behavior of plants in a soil of this type, in which the phosphate is held either by kaolinitic or iron colloids, or by both? The time factor must be important, and the trees have, at least under California conditions, a long annual period for phosphate absorption and

<sup>9</sup> Private communication, Dr. O. L. Lilleland.

also have the potentiality to develop extensive root systems. It is conceivable that there are other influential factors related to specific root activities. Though colloids of the type now under consideration do not release phosphate to mineral acids of a strength of biological significance, phosphate can be released by certain organic acids. If, contrary to the usual view, suitable organic acids in sufficient amounts could be excreted by roots, or perhaps even enter the soil through root decay, the effect on the ability of the plant to obtain phosphate might be a factor of consequence. Unfortunately at present this is largely a matter of speculation.

Another, and in many areas, more important type of soil system than the one just discussed is that in which phosphate solubility is governed by calcium compounds. Buffering effects and the power of the root system to cope with them become of great significance (cf. 7). Finely ground rock phosphate as a source of phosphate for plants has been studied for many years, and the familiar features of differential plant response to a calcium phosphate system of this kind will be recalled. The theory of a relation of calcium to phosphate absorption is not an adequate explanation of different plant responses to slightly soluble phosphates, since there is no generally consistent relationship between the adequacy of the phosphate medium and the inherent power of some plants for large calcium absorption. This point is illustrated by comparisons of buckwheat and tomato plants; both have potentialities for high calcium absorption but they differ in their response to rock phosphate. Though the problem is an old one, more definite information is still needed on the relative rates of respiration and active nutrient absorption by the roots of plants with different responses to phosphate in the medium, on relative extent of root surface and efficiency of root contacts with mineral surfaces, and on comparative phosphate requirements for growth of different plants in relation to the time factor. We are, of course, referring only to the physiological side of the problem without discussion of the interrelated questions of soil chemistry to which important contributions have been made in recent years.

#### CHEMICAL AND BIOLOGICAL TESTS OF AVAILABILITY

It has become increasingly obvious that no one chemical criterion can be invoked in appraising the supplying power of all soils for a specific nutrient element. A case in point is that of potassium availability in soils. At one time it was rather widely accepted that replaceable potassium, i.e., potassium displaced from the soil colloid complex in the process of base exchange, is a measure of available potassium. That this view is no longer tenable for many soils is illustrated by the following experiment:

A 7-year greenhouse study was made of the absorption of potassium by barley and tomato plants from a series of California soils of great diversity with respect to their ability to supply potassium to the plants mentioned (9). One of these soils had a relatively high content of replaceable potassium, that is, several hundred parts per million of the dry soil, at the beginning of the experiment, and for some time there was no response to additions of potassium. When,

however, the replaceable potassium of the soil had become depleted to a minimum value by crop growth, striking evidence of physiological potassium deficiency appeared. Other soils had a low content of replaceable potassium initially and soon failed to supply adequate amounts of potassium. But still other soils with similarly low contents of replaceable potassium showed little or no deficiency even when intensively cropped for a large part of each year over a period of 3 to 4 years. Additions of nitrogen or phosphate were made to the soils to prevent deficiencies of these nutrients.

Potassium was supplied by some of the soils to the crop from nonreplaceable potassium, in the sense that amounts of this element equivalent to those absorbed by the plants were not recovered in leachings of the soil with ammonium acetate or dilute acids. In recent years numerous investigators have obtained similar results, as did some who worked a long time ago in less extensive researches. As an illustration, several workers have made a survey of a great number of California soils by comparing Neubauer values and replaceable potassium values. Sometimes there is approximate agreement, but there is no general consistency between the two values. In many instances the amount of potassium absorbed by the rye seedlings in the Neubauer procedure was much greater than the amount of replaceable potassium as determined by the usual laboratory method. The various "quick" tests likewise failed to show a consistent relation to the Neubauer values. In the vegetation experiment referred to, potassium deficiencies were much better correlated with Neubauer values than with chemical tests. In other words, in numerous soils studied, the chemical reagents likely to be employed in ascertaining availability of potassium do not reflect correctly physiological conditions.

In one experiment with a soil of good potassium-supplying power, as indicated physiologically, a comparison was made between the Neubauer potassium value and the amount of potassium obtained by 10 days of continuous leaching of the soil with saturated carbonic acid. The Neubauer value was much larger for this soil. Some of these facts are not easily explained. Perhaps the contact effects already referred to will eventually give further understanding of these phenomena, but on the other hand, in the contact experiments, colloids containing adsorbed bases removable by leaching with appropriate reagents, and so "replaceable" in the present usage of the term, were used (13). Regardless of the explanation, the properties of the biological system, especially as represented by the roots, must be taken into account. The rapid absorption of potassium by the plant will selectively disturb the equilibrium of the soil system in a way not so far adequately imitated in the laboratory. This disturbance of equilibrium will have important consequences whatever the nature of the first step in the absorption of potassium by plants.

Some recent direct evidence on the absorption of a nonreplaceable base by plants was obtained by employing a relatively long-lived radioactive rubidium isotope added to two soils of high fixing power for potassium.<sup>10</sup> In the particular soils studied, much of the fixed rubidium was present in nonreplaceable

<sup>10</sup> Unpublished data of J. C. Martin and R. Overstreet.

form. Seedling barley plants, however, removed significant amounts of the rubidium which was not previously extracted by the leaching reagents. Since the rubidium ions were tagged there could be no doubt that the ions withdrawn by the plants came from those originally added to the soil.

These as well as some other observations on absorption of potassium and other ions discussed previously (4, 13) were made with the aid of the new tool of radioactive isotopes. One of the chief advantages of this technique is the possibility of following experimentally small newly introduced amounts of "tagged" atoms without reference to the large stores of an element already present in a living organism or in a nutrient medium. Although the use of this method is sometimes more a matter of convenience than of indispensability, it is interesting to point out that only by experiments with isotopes can certain facts be established. As one specific case, it becomes possible by employing radioactive isotopes to measure outward movement of an ion from healthy, actively metabolizing roots at the same time that net accumulation of the same ion species is occurring (6). This can be done by allowing roots to absorb a limited amount of labelled ions and then placing them in a solution (or colloid suspension) containing the same ions in nonradioactive form. By following this procedure, one can measure the net ionic movement by methods of total analysis and that of the radioactive ions by determining the radioactivity of the tissue or culture solution.

While discussing the general subject of soil and plant interrelations with respect to potassium, we wish to recall the observation that during prolonged cropping, in the pot studies previously cited, some of the added potassium became so firmly fixed, in certain of the soils, as not to be appreciably available to plants. Whether there were special conditions of wetting and drying under the experimental circumstances studied which were conducive to the fixation of potassium in difficultly available form is still undecided.

#### COMMENTS ON PRACTICAL METHODS FOR ASCERTAINING NUTRIENT DEFICIENCIES

It has seemed necessary in the preceding discussion to stress the complexities of the subject under consideration. It is recognized, however, that immediate answers to practical questions may need to be sought by short cuts, without awaiting the results of long-time researches. There are two general types of procedures in survey work; soil analysis and biological tests, and both have their necessary functions. On behalf of biological tests, it may be said that the plant itself gives an integration of the exceedingly complex system of soil and plant. The idea of analyzing plant tissues in the study of nutrient deficiencies is a venerable one, but we gain the impression that there is a renewal of interest in this approach. Leaving aside the more complex investigations on foliar diagnosis, brief reference may be appropriate to simple methods of diagnosis, as illustrated by studies on potassium. It is evident from the work of many investigators that the plant tissue content of potassium may be greatly influenced by the nutrient medium. For example, in the experiments

with barley and tomato plants already described there was a high correlation between percentages of potassium in the dried vegetative tissues and the response of the plant to potassium fertilization.

The possibility exists also of sometimes obtaining useful indications of potassium-supplying power of soils from analysis of samples of plant tissue taken at suitable stages of growth from crops growing in the field. According to some investigations, including one being carried on in California, the analysis of petioles, for some kinds of plants, gives an especially valuable reflection of soil conditions with reference to potassium. But climatic, physiological, and soil factors modifying the interpretation of results should not be disregarded. In the interpretation of petiolar values for potassium, the amounts of other bases present may need to be taken into account. In this connection, sodium is important in many arid soils. Also, as suggested earlier in this article, the requirement of potassium by the plant in relation to heaviness of fruiting has a most important bearing on the conclusions to be drawn from analysis of leaf tissues. Studies on prune trees in some sections of California have provided a striking illustration of this point. Other difficulties with respect to interpretation of phosphate relations on the basis of analyses of leaves of fruit trees have been noted (14).

This rapid survey has been undertaken primarily to stress the physiological aspects of the general soil-plant problem under discussion. There is nothing new in this point of view, but we have often been impressed, in reading discussions of availability of nutrients and descriptions of laboratory methods, by the apparently insufficient attention given to complex physiological interrelations of plant, soil, and climate. Even though empirical methods of appraising nutrient deficiencies in the soil may be devised for a particular soil-crop-climate system, we feel justified in suggesting that further intensive study, not only of the soil system, but of the active role played by the plant as well, will assist in the development of a more satisfactory understanding of both theory and practice. If the plant physiologist and the soil chemist often disappoint the farmer who seeks a simple procedure in assessing the fertility of his soil, they may plead that the complexity of plant-soil interrelations is not one of their own making, but that it is rather inherent in the nature of the systems.

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# THE BALANCE OF IONS AND OXYGEN TENSION IN NUTRIENT SUBSTRATES FOR PLANTS<sup>1</sup>

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## THE BALANCE OF IONS IN NUTRIENT SUBSTRATES

There are two general concepts concerning the balance of ions in nutrient substrates for plants. The first of these proposes that exact proportions of ions in a nutrient substrate for plants are not particularly important, or that exact balance can have little significance with respect to plant responses. As long as no actual deficiency of any of the essential nutrient ions occurs, the solution is considered satisfactory. The second concept is that for any given set of factors external to the nutrient substrate there can be only one set of proportions of the essential ions in the nutrient substrate to which the plant will respond appropriately or in a definitely prescribed manner.

In accordance with the first concept, plants will grow and develop equally well within a relatively wide range of ion proportions and within a restricted range of total concentration of the essential ions. This method of approach to the problem has its advantages but also some very obvious disadvantages. In the practical application of ion balance to large-scale plant production or in any agricultural system of fertilizer practice, however, this is about the only method of approach at present available. Under this scheme an accepted standard nutrient mixture may be selected and utilized, the purpose for which it is utilized determining the proportions of the ions in the mixture. In such a nutrient substrate, be it a culture solution, a sand culture to which the culture solution is applied, or an acre-foot of soil to which is applied a standard fertilizer mixture, the balance of the ions will gradually undergo changes under the influence of growing plants, but it is assumed that as long as no actual deficiency of any one ion occurs, such change of balance can have no appreciable effect upon the responses of the plants. This is a very comforting idea, particularly to those who are engaged in quantitative experimental investigation. Moreover, it is a well-known fact that the proportions of the nutrient ions in a substrate may be altered through a relatively wide range without appreciably affecting the plant if the criteria for rendering a judgment are of a certain type, such as rates of growth, linear measurements, or weight production of plant material. If the criteria for rendering a judgment are based, however, upon

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a careful quantitative examination of the chemical make-up, or even upon the morphological characteristics of the plant, then the general principles upon which the concept is based will no longer be valid. It has been shown repeatedly that alterations in the balance of ions of the nutrient substrate, and in the case of some ions even a slight alteration, will result in a pronounced alteration in the quality, that is, in the chemical and physical constitution, of the plant product.

This first general point of view with respect to the balance of ions in nutrient substrates lends itself admirably to the practical application of the general principles of plant nutrition in agricultural practices, and it is well adapted also to the procedures in certain of the less refined and less exacting types of experimental plant studies. It is more expedient, however, than ideal in its application. It is not born of the true scientific method, but with our present limited knowledge about nutrient ions and their balance, this general point of view is an absolute necessity for the practical application of those few well-known general principles of plant nutrition which have been so helpful not only in the production of plants on a commercial basis but also in the scientific study of them.

The second general concept concerning the balance of ions in nutrient substrates should be regarded perhaps as ideal rather than as practical. It is obvious, according to this general point of view, that in order to maintain the so-called appropriate response, every change occurring in any one of the environmental factors that can influence the plant, such as light intensity, temperature, and humidity, must be accompanied by a corresponding shift in the balance of ions to compensate for or to counteract the disturbing effect of the variable factor. This point of view, as may be seen readily, is exacting. Every factor involved must be defined with mathematical precision, but this is the scientific approach to the problem of ion balance in nutrient substrates. It is an ideal toward which to strive in the investigation of any problem in which the physiological balance of ions in nutrient substrates for plants is involved. It is a scientific ideal, however, which with our present knowledge, is impossible of attainment, even under the most rigid system of controls, because it is not possible as yet to isolate all the factors that influence the plant and to deal with them appropriately.

In accordance with this second general concept, the approach to the problem must always be an attempt to approximate the ideal as closely as possible, and in certain types of investigations involving the balance of ions, this is the only possible course to pursue if the objective of the investigation is to be attained. As examples of the type of problems which demand this method of approach are the problems in trace element nutrition of plants, as for example, a study of the role of boron in calcium metabolism, or a study of the interrelationships between the elements iron and manganese in the physiological processes in which these elements function. The general concept or point of view first mentioned would be utterly useless as a method of approach to problems of

this nature. In problems of this kind the nearest possible approximation of the ideal in the experimental set-up, in the purity of salts and of distilled water, in cleanliness of apparatus involved, and in the balance of ions and their concentration in the substrate is absolutely indispensable if the objectives of quantitative research are to be attained.

Each of these concepts or points of view demands a prominent place in the experimental procedures in the field of the plant sciences. The first is adaptable to, and more generally useful in, the investigation of problems relating to the applied plant sciences. The second is the only effective method of approach to the investigation of fundamental principles involved in the nutrition of plants as well as other fundamental problems concerning the physiology of plants. It should be emphasized, perhaps, that the balance of ions in a substrate for plants is much more closely associated with the quality of the plant or plant product than it is with rates of growth and yields. This fact provides a criterion for suggesting the method of approach to problems involving the proportions of nutrient salts or the balance of ions in nutrient substrates.

Furthermore, it cannot be too strongly emphasized that, within reasonable limits, it is not the intrinsic quantity of any essential element, either in the substrate or in the plant, which is important, but it is the relation of this quantity to the quantities of other elements which is significant and which establishes that delicate dynamic balance of the elements so essential for well-regulated growth and development. It is particularly this balance in its chemical constitution which determines the quality of the plant and of its products. The balance of ions in a nutrient substrate for plants cannot attain its maximum effectiveness, however, without an adequate supply of an element which, up to the present time, has been given but slight consideration as an important factor in the nutrition of a plant. That element is oxygen.

#### OXYGEN IN NUTRIENT SUBSTRATES

A culture solution for plants containing, in proper proportions and in suitable concentrations, all the mineral elements usually considered essential for growth and development does not constitute an adequate inorganic nutrition system without the presence of dissolved oxygen. Important as this factor is in the absorption and accumulation of nutrient ions and in the vital plant processes in general, adequate consideration of its importance has been singularly neglected in studies dealing with the mineral nutrition of plants.

The disastrous effect of an inadequate oxygen tension in a nutrient substrate upon growth can readily be demonstrated experimentally. Plate 1 shows the effect of different rates of aeration on the growth of soybean plants in solution culture. Each 1-gallon jar contains the roots of three soybean plants. The culture solution was supplied by the continuous flow method at a rate sufficiently high to avoid any pronounced change in either the pH of the culture solution or the balance of the nutrient ions as influenced by the growing plants. Culture 1 received no artificial aeration, although no attempt was made to

exclude atmospheric air from the surface of the solution. Cultures 2, 3, and 4 were aerated by thrusting one capillary tube to the bottom of culture 2, two capillary tubes to the bottom of culture 3, and four capillary tubes to the bottom of culture 4, and forcing air under constant pressure through all the tubes. All the capillary tubes were equal in length and had approximately the same bore; therefore, if culture 2 be regarded as having received unit rate of aeration, culture 3 received unit rate multiplied by 2, and culture 4 received unit rate multiplied by 4.

It may be observed that the roots of the nonaerated culture made very poor growth as compared with the aerated cultures. The roots of culture 1 were brown, whereas those of the aerated cultures were silvery white. The poor growth of roots in culture 1 was reflected also in the growth of the tops. Though the growth of roots in all the aerated cultures was excellent, best growth occurred in culture 4, and this also was reflected in the growth of the tops.

The graphic record of oxygen tensions in the solutions of the four cultures carried out in triplicate over an experimental period of 24 hours is given in figure 1. The average oxygen tensions, determined at intervals over the 24-hour period, are plotted against time. As indicated by the graphs the initial oxygen tension of all the culture solutions was the same and was somewhat below the saturation point at equilibrium with the atmosphere. The average oxygen tension of the solutions receiving the 4-unit rate of aeration quickly rose to approximately the saturation point, that of the solutions receiving the 2-unit rate of aeration was maintained at approximately its initial concentration, but the average oxygen tension of the solutions receiving the unit rate of aeration was quickly reduced to a value considerably below the initial concentration by the action of the plants. The most striking feature of this record, however, is the rapid reduction of the oxygen tension of the culture solutions by the action of the plants of the nonaerated cultures. The graph representing the average data for these cultures shows that the oxygen concentration was reduced to nearly the zero point in 6 to 9 hours in spite of the fact that 120 square centimeters of solution surface were continuously exposed to the oxygen of the air. The growth produced by these cultures as indicated by the average yield values of both roots and tops is directly correlated with the aeration rates; that is, average low yields correspond to low oxygen tension in the nutrient substrate, and average high yields are definitely associated with high oxygen tension.

As indicated by the graphs in the upper part of figure 1, the oxygen tension in the solutions of the aerated blank cultures rose quickly to the saturation point in equilibrium with the atmosphere, while in the nonaerated blanks the initial oxygen tension was approximately maintained. Fluctuations in the graphs are due to the fluctuations in the temperature over the 24-hour period, as indicated by the temperature curve in the lower part of the figure.

Important as an adequate supply of dissolved oxygen in the nutrient substrate is for growth and development of the plant, the effect upon growth as

such is not direct but is intimately associated with the complex of processes which result in growth and development, including the processes involved in inorganic nutrition, such as the absorption, accumulation, and assimilation of the nutrient ions. This was clearly brought out in a quantitative study of these processes in plants grown in a standard nutrient solution at different oxygen tensions that were maintained at approximately constant levels over prolonged experimental periods, subject, however, to variations due to changes in greenhouse temperatures, which were not controlled within very narrow limits.

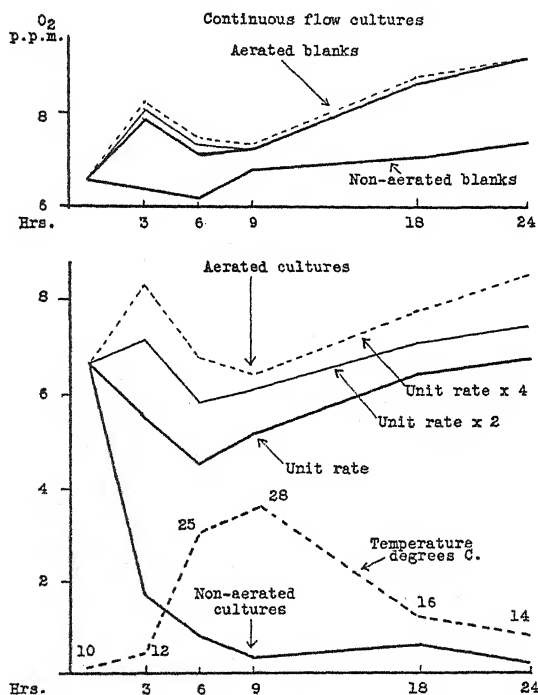


FIG. 1. EFFECT OF SOYBEAN ROOTS ON THE OXYGEN TENSION OF AERATED AND NONAERATED CULTURE SOLUTIONS

In these studies three species of plants were employed, soybeans, oats, and tomatoes. The plants were grown in standard culture solutions in which concentrations of dissolved oxygen were maintained at approximately 0.0, 4.0, 8.0, and 16.0 p.p.m. This was accomplished by aerating the solutions with the proper mixtures of oxygen and nitrogen to give the oxygen concentrations in question when these were in equilibrium with the confined gas mixtures maintained over the solution surface. A special cultural technique permitted such confinement as well as isolation of the root systems of the intact plants out of contact with the external atmosphere. This was accomplished as

follows: the radicles of the seedling plants, while still very young, were thrust through glass tubes fixed, flush with the upper surface, in the large, flat rubber stoppers which sealed the wide mouth of the culture jar. The glass tubes, holding the seedlings, were just long enough to project from the stopper, 2 cm. under the surface of the solution below, thus providing an effective water seal so that the gas space between the rubber stopper and the solution surface was completely isolated from direct contact with the atmospheric air. No attempt was made, however, to exclude air from the very small solution surface exposed between the plant stems and the inner walls of the glass tubes. This surface was negligible from the standpoint of supplying oxygen to the nutrient substrate, since the stems of the plants, when they had attained the age and size required for experimental use, virtually closed the glass tubes. Tubes of appropriate size were selected with this point in view. With this system the oxygen tension in the substrate could always be maintained at approximate equilibrium with the oxygen pressure of the gas mixture used for aeration at the prevailing temperature. Gas inlet and outlet tubes in the rubber stoppers provided facilities for aeration of the culture solutions and for collecting the effluent gasses for analysis. The roots of the plants projected into the culture solution from the lower end of the glass tubes, and the tops were exposed to the atmosphere of the greenhouse.

The experimental plants were grown in the standard culture solution until they had attained the desired stage of development before they were transferred to the oxygen-treated cultures. The rates of absorption of ions from solution during the test intervals were determined by the usual technique of solution analysis before and after contact with the root systems. At the end of the test interval, which also terminated the experimental period, the plants were harvested, dried, and weighed in preparation for chemical analysis of the tissues. All of the data considered in this report were obtained from plants of cultures which had been subjected to the various oxygen treatments during an experimental period of 20 days. Prior to the experimental period all cultures received identical treatment.

#### *Oxygen requirement*

The oxygen requirement of the plants as determined by the effect of oxygen tension in the nutrient substrate upon dry weight yields of plant tissue was markedly different for the three species studied. An approximate optimum requirement around 6 p.p.m. of dissolved oxygen in the substrate was indicated for soybeans. That is, average dry weight yields increased with increasing oxygen concentration in the substrate up to 6 p.p.m. Above this range the soybean plant showed definite symptoms of toxicity, as indicated by a characteristic distortion of newly formed leaves in the process of expansion and by a dry weight yield considerably below that of the maximum average yield produced at an oxygen concentration of 6.0 p.p.m.

The oxygen requirement of the oat plants as determined by the dry weight

criterion showed an optimum around a concentration of 8.0 p.p.m. in the nutrient substrate. At a concentration of 16.0 p.p.m. the average yields obtained were considerably lower than they were at a concentration of 8.0 p.p.m.

The oxygen requirement of tomato plants appears to be remarkably high, judged by the effect of oxygen concentration in the substrate upon the dry weight production of young plant tissue. The highest average dry weight yields were obtained with an oxygen concentration of 16.0 p.p.m. in the nutrient substrate. Whether still higher yields could have been obtained with higher oxygen tensions was not determined.

The general conclusion to be drawn from the dry weight yield data with respect to the effect of oxygen concentration in the substrate upon growth as such is that yields may be expected to increase with increase in the oxygen concentration maintained in the nutrient substrate up to an optimum which varies widely for the different species. It is further indicated that a nutrient substrate saturated with oxygen at the equilibrium point with atmospheric air is considerably below the optimum required for maximum yields of some species. This consideration may be of great practical importance from the standpoint of agricultural production.

*Effect of oxygen in the nutrient substrate upon absorption, accumulation, and assimilation of nutrient ions*

This preliminary report on the phase of oxygen studies here dealt with presents data for only one species, the soybean, and for only one nutrient element, nitrogen. Similar studies with oat and tomato plants have not yet been completed, but the results thus far obtained with these two species are similar to those obtained for the soybean with only such minor variations as might be expected because of structural differences. The soybean plants were grown during a treatment period of 20 days in culture solutions, continuously renewed, with oxygen concentrations maintained at approximately 0.0, 4.0, 8.0, and 16.0 p.p.m. At the end of the 20-day treatment period the rates of absorption of nitrogen in both the anion and the cation form were determined for an absorption interval of 9 hours in terms of milligrams of nitrogen absorbed per gram of dry root tissue per hour. These average data for the nitrate ion plotted against the oxygen concentrations of the nutrient substrate are presented in figure 2.

It will be observed that the highest rate of nitrate-nitrogen absorption occurred in the culture with the lowest oxygen. Figure 2 shows a rapid decline in the rate of nitrate-nitrogen absorption for each increase of the oxygen tension in the nutrient substrate, the highest rate corresponding to the lowest oxygen tension, and the lowest rate corresponding to the highest oxygen tension. The broken line in figure 2 represents the nitrate nitrogen, in terms of milligrams of nitrogen per gram of dry tissue, which had accumulated in the roots of the plants as determined at the end of the experimental period. These average values, shown as ordinates on the right, are also plotted against the



oxygen concentrations of the nutrient substrate. The graph shows the lowest nitrate-nitrogen accumulation value in the roots of the plants grown at the lowest oxygen tension. The values rise sharply to a maximum at an oxygen tension of 4.0 p.p.m., and then slightly decline with further increase in the oxygen tension of the nutrient substrate. Thus low accumulation of nitrate-nitrogen is shown to correspond to a high rate of absorption; and high accumulation, to low rates of absorption. This relation between accumulation and the rates of absorption of the nitrate ion, at first consideration, may appear to be contradictory. It may be explained, however, by assuming that the rate of nitrate reduction in the roots of the plants is high at low oxygen tension, when the rate of absorption also is high, and that the rate of nitrate reduction is low

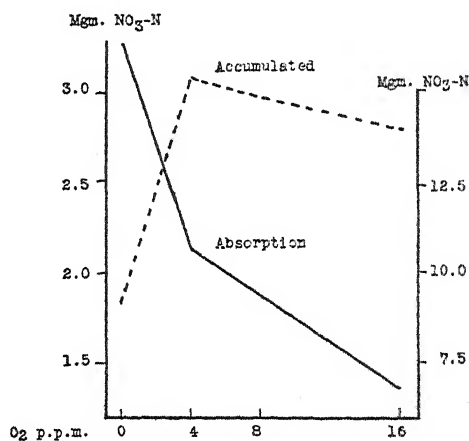


FIG. 2. RATES OF NITRATE-NITROGEN ABSORPTION IN MILLIGRAMS PER GRAM OF DRY ROOT TISSUE PER HOUR (LEFT), AND NITRATE-NITROGEN ACCUMULATED, PER GRAM OF DRY ROOT TISSUE (RIGHT), AT DIFFERENT OXYGEN LEVELS

when the rate of absorption is low, at high oxygen tension in the nutrient substrate. That this is precisely what does occur is indicated in figure 3.

The data in figure 3 represent the percentage of the total nitrogen present in the root tissue as nonnitrate nitrogen at the end of the experimental period, plotted against the oxygen concentration of the nutrient substrate. Since all the nitrogen present in the roots was absorbed in the form of nitrate, these values represent that portion of the total nitrate nitrogen absorbed which was reduced and transformed into other compounds of nitrogen in the metabolic processes of the roots. This fraction, therefore, provides an index of the nitrate reduction by the root tissue as affected by oxygen concentrations of the substrate.

It will be observed from figure 3 that this index of nitrate reduction is highest at the lowest oxygen tension of the substrate and that it is low at the high oxygen levels. A comparison of this graph with that of figure 2 representing

nitrate absorption shows the general similarity of the two graphs, indicating that nitrate absorption and nitrate reduction are similarly related to the oxygen supply of the substrate. This accounts for the apparent contradiction that the accumulation of nitrate nitrogen is highest when the absorption rates are lowest. It is apparent also that the high accumulations of nitrate nitrogen in the roots, such as are indicated in figure 2 under conditions of high oxygen levels in the substrate, can occur only when the rates of absorption greatly exceed the rates of reduction.

During these studies it was commonly observed that the soybean plants grown in solutions with deficient oxygen, even those grown at the lowest oxygen level (0.0 p.p.m.) could persist, with growth rates much below the optimum, for considerable periods and could even reproduce under these conditions, although very poorly. Certain evidence strongly suggests that this ability to

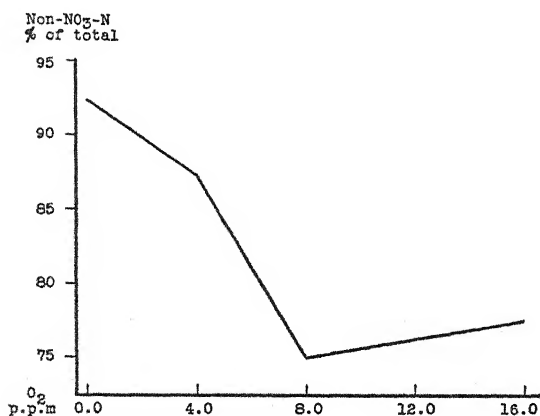


FIG. 3. NONNITRATE NITROGEN IN PERCENTAGE OF TOTAL, AS INFLUENCED BY THE OXYGEN LEVELS OF THE SUBSTRATE: AN INDEX OF NITRATE REDUCTION IN SOYBEAN ROOTS

persist and reproduce without an adequate oxygen supply in the substrate is directly related to the reduction of nitrates in the root tissues and the utilization of the oxygen evolved in this process. The evidence suggests that the oxygen evolved in nitrate reduction is utilized in the respiratory processes of the root cells, and this is emphasized by the fact that the soybean with a relatively low oxygen requirement is much less severely affected by these semi-anaerobic conditions than are oat and tomato plants, which have a relatively much higher oxygen requirement than the soybean. That oxygen evolved in the reduction of nitrates is utilized in the respiratory processes is indicated by the fact that nitrate reduction is abnormally high only in those plants grown in an oxygen-deficient substrate. This also accounts for the high rate of absorption under these conditions. It is further indicated by the fact that when one-half the nitrate nitrogen in the substrate is replaced by a molar equivalent concentration of ammonium nitrogen, the plants are quickly and

much more severely affected by oxygen deficiency in the substrate than they are with an adequate supply of nitrate-nitrogen under the same conditions. With an adequate supply of oxygen in the substrate the plants are not at all adversely affected by this substitution of ammonium nitrogen for nitrate nitrogen.

The effect of the oxygen supply of the substrate upon the rates of absorption and accumulation of cation nitrogen ( $\text{NH}_4^+$ ) presents a picture which is the exact opposite of that shown for the absorption and accumulation of anion nitrogen ( $\text{NO}_3^-$ ). This is clearly brought out in figure 4 which shows the average rates of absorption of ammonium nitrogen in terms of milligrams per gram of dry root tissue per hour, for an absorption interval of 9 hours, plotted against the approximately maintained oxygen concentrations of the nutrient substrate. These tests were carried out with both nitrate nitrogen and ammonium ni-

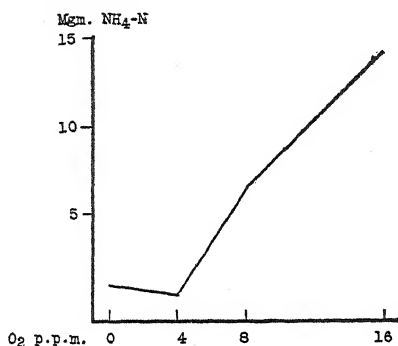


FIG. 4. RATES OF AMMONIUM-NITROGEN ABSORPTION, IN MILLIGRAMS PER GRAM OF DRY ROOT TISSUE PER HOUR, AT DIFFERENT OXYGEN LEVELS

trogen present in the culture solution in approximately molar equivalent concentrations. The graph shows very low absorption rates at low oxygen levels. The rise in these rates is rapid as the oxygen concentrations of the substrate increase, the highest rate corresponding to the highest oxygen tension. This appears to be the general effect of the oxygen supply in the nutrient substrate upon the absorption and accumulation of both cations and anions, as has been pointed out by Hoagland and Broyer (3). These authors make it clear that the accumulation of salts is associated with active aerobic respiration and that an adequate oxygen supply is indispensable for both cation and anion accumulation. Prevot and Steward (4) also point out that cells which are most actively engaged in the accumulation of ions show the greatest affinity for oxygen. It is to be emphasized strongly, however, that the absorption of ions containing oxygen which can be rendered available and utilizable in anaerobic respiratory processes in the cells may alter completely this general relation between the oxygen supply of the substrate and the rates of absorption and accumulation of ions by the roots of plants. This particular phase of the

oxygen relations may have a very important bearing upon agricultural production, since it is readily conceivable that the roots of plants in many agricultural soils may be growing under oxygen tensions of the soil solution far below the optimum required for good growth.

*Relation of the oxygen tension of the substrate to the organic acid content of plants*

Evidence that the supply of oxygen in the nutrient substrate for plants has far-reaching effects, not only upon absorption, accumulation, and assimilation of ions, but also upon the general metabolic processes of the plant, is indicated

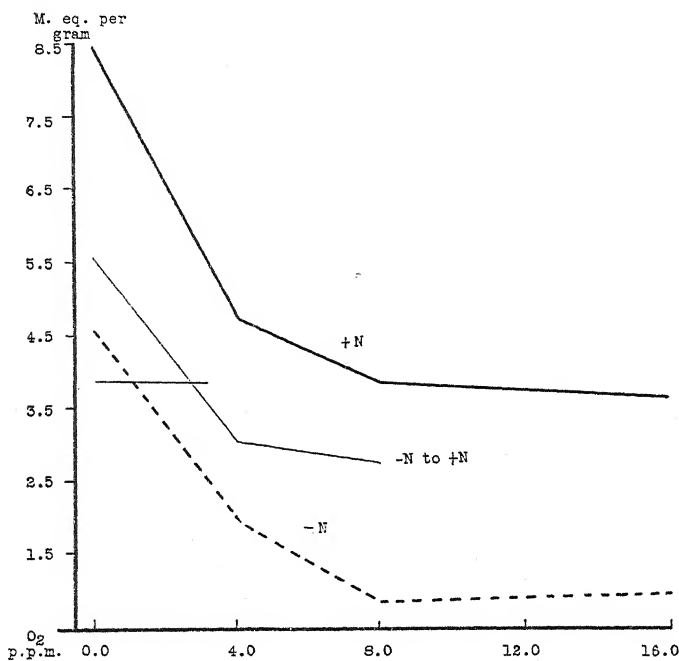


FIG. 5. THE ORGANIC ACID CONTENT IN TERMS OF MILLIEQUIVALENTS PER GRAM OF DRY SHOOT TISSUE OF OATS AT DIFFERENT OXYGEN LEVELS

Upper graph, plants grown 6 days in a +N substrate. Middle graph, plants transferred from a -N to a +N substrate for 24 hours. Lower graph, plants grown 6 days in a -N substrate. The short horizontal line intersecting the broken line represents the average value of the organic acid content of the plants at the beginning of the treatment period and may be regarded as the organic acid content of these plants under optimum growth conditions with respect to the oxygen supply.

by the data relating to organic acid production presented in figure 5. These preliminary studies were carried out with oats as the indicator plants. The plants were grown in a standard culture solution by the usual cultural methods until they had attained the desired stage of development. They were then transferred to culture solutions at the maintained oxygen tensions indicated

in figure 5 and were grown in these solutions during an experimental interval of 6 days. Two series of plants were grown: one series with nitrate in the culture solution as the source of nitrogen for the plants; the other, without nitrogen. The plants of the two series will be designated "plus-nitrogen" and "minus-nitrogen," respectively. Plants were analyzed for total organic acids both at the beginning and at the end of the experimental period by the methods of Pucher, Vickery, and Wakeman (5, 6). The data, plotted against the oxygen concentrations of the nutrient substrate, are presented in figure 5.

It will be observed from the graphs of figure 5 that the highest organic acid content of the plants in each series occurs at the lowest oxygen level and decreases with increase in the oxygen level to the point of the optimum oxygen concentration of the substrate for this species. At this point the graphs level off to the horizontal, showing that further increase in the oxygen tension has no effect upon organic acid production. It may also be observed that the organic acid content of the plus-nitrogen plants is more than double that of the minus-nitrogen plants at each oxygen level. It is interesting and perhaps important also to note that the total organic acid content of the plus-nitrogen plants at suboptimal oxygen levels (below 8.0 p.p.m.) is, without exception, higher than the normal, and that of the minus-nitrogen plants, except at the lowest oxygen level, is very much lower than the normal. It is thus evident that the nitrogen supply is also a controlling factor in the production of organic acids in the plants. In these studies, however, it was not determined whether the high organic acid content of the plus-nitrogen plants, relative to that of the minus-nitrogen plants, was due to the presence of nitrogen as such, or was directly related to the absorption of the nitrate ion and its ability to act as an oxygen donator in the respiratory processes under conditions of oxygen deficiency in the substrate. On this point Clark (2) has provided evidence which shows that plants grown with nitrate as the source of nitrogen produce much higher yields of total organic acids than do the same plants grown with ammonium as the source of nitrogen. Blackman and Templeman (1) also have shown that the exceptionally high yields of total organic acids by plants are significantly correlated with the accumulation of nitrates. The very high yields of total organic acids by the plus-nitrogen plants at the lowest oxygen level in the nutrient substrate definitely associate these with anaerobic conditions and, therefore, with high rates of nitrate absorption and reduction, as previously shown.

At the end of the experimental period of 6 days some of the minus-nitrate plants were returned to a plus-nitrate substrate for 24 hours. There was an immediate rise in the total organic acid content of the plants, as is indicated by the middle graph of figure 5. Though this rise is still controlled by the oxygen tensions of the substrate at concentrations below that of the optimum (8.0 p.p.m.), it is quite apparent that nitrogen, particularly when it is present in the form of the anion, may exert a powerful influence not only upon the role which oxygen plays in the origin of organic acids but also upon the entire metabolic system of the plant.

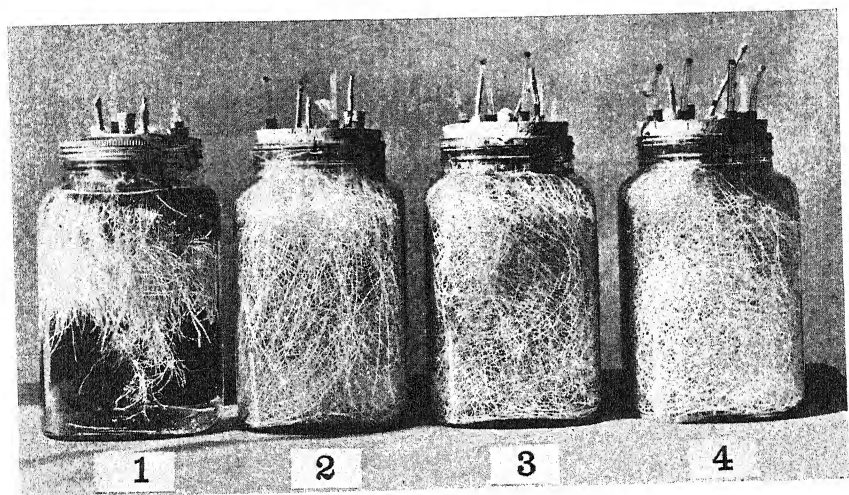
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## PLATE 1

## EFFECT OF AERATION ON GROWTH OF SOYBEAN ROOTS

1. Nonaerated. 2. Unit rate of aeration. 3. Unit rate multiplied by 2. 4. Unit rate multiplied by 4.







## ION AND PLANT RELATIONSHIPS IN WESTERN ARID SOILS

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As one goes westward from the Mississippi River, he traverses land of decreasing annual rainfall and reaches a zone of black soil extending almost straight north and south, from Canada to the Gulf. In this zone of chernozem soils rainfall is sufficient for the growth of good crops of grasses and of the cereal grains such as wheat. The western edge of this belt marks the beginning of arid soils. It coincides fairly well with the 100th meridian, which is often taken as the eastern boundary of the arid West. To fix the location in your mind, the 100th meridian forms the east-west boundary between the Texas Panhandle and Oklahoma and it lies 2 degrees east of the Colorado-Kansas boundary. I would include in the arid West all land between this meridian and the Pacific Coast except a region west of the Cascades in Washington, Oregon, and California where the rainfall is high. The rainfall of the arid West is less than 20 inches per annum in general, and less than 10 inches annually over extensive areas (fig. 1).

### BASIC DIFFERENCES BETWEEN ARID AND HUMID SOILS

In order to understand plant relationships on arid soils, let us first briefly review a few salient differences between arid and humid soils. A more thorough review of western soils and their development is given by Kellogg (8) in his discussion of the great soil groups of the United States.

The major difference between western and eastern soils lies in the presence of an accumulation zone of calcium carbonate. Western soils, with such an accumulation, are called "pedocals," as opposed to the acid soils of the East, called "pedalfers." In the East, with more abundant rainfall, the soil water becomes laden with carbon dioxide, and calcium and magnesium compounds are dissolved and carried into the ground waters as bicarbonates. The replaceable bases are also in part removed from the clay fractions, thus forming acid soils. Under arid conditions, more of the water evaporates from the soil surface or is lost by transpiration, and any dissolved calcium and magnesium salts are likely to be deposited in the subsoil as carbonates. The soil colloids are fully saturated with bases.

In the East, percolating rain waters carry downward the finely divided clay particles. This process is called "podzolization." Under western arid conditions the colloids remain flocculated because of the neutral or alkaline

soil reaction, and because of this condition as well as the scarcity of the transporting agent (water), very little colloid is carried downward.

Under a climate noted for its lack of precipitation, hot summers, and hot days with cool nights, the physical types of weathering have been accentuated as compared with the East. We have, therefore, sands which are so classified from the standpoint of grain size but which may not be high in silica content. Though some chemical weathering has occurred in the West, it is less pronounced. During the weathering processes, salts are formed, but they tend to accumulate for lack of leaching water to dissolve and carry them away.

Let us look further into this question of rainfall. The annual average for the West is less than 15 inches. It comes in a few storms, and because of sparse vegetation and hard uncultivated ground, runoff is great—probably 50 per

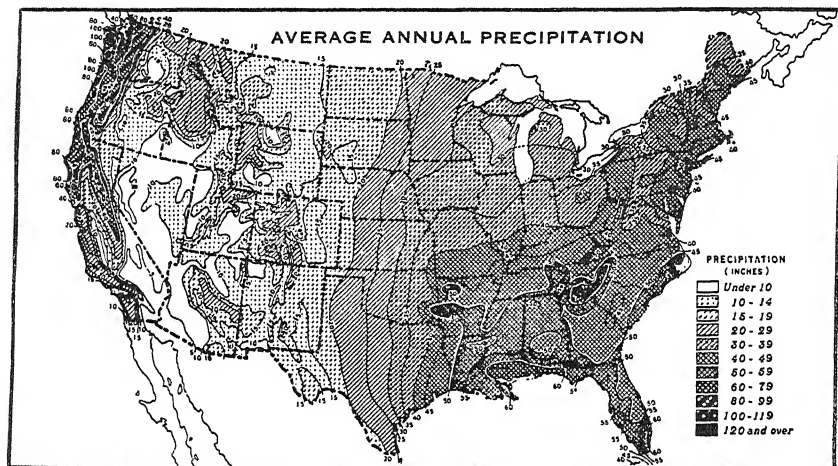


FIG. 1. AVERAGE ANNUAL PRECIPITATION OF THE UNITED STATES

(From U. S. Dept. Agr. Misc. Bul. 260)

cent. The remainder soaks in. The ground may be wet to a depth of 15 inches, if several rains follow at short intervals. Thus, at Mandan, North Dakota, on the eastern fringe of our arid soils, with an annual rainfall of 15.2 inches, water has penetrated beyond the depth of wheat roots only once in 25 years, according to Cole and Mathews (4), and then only under a precipitation not duplicated during the period 1878 to 1936. Under such conditions the surface one or two feet are charged with water annually. The water is removed annually by evaporation and vegetation, and the vegetation is limited by the amount of water present. The deeper soil horizons are always dry. Under these conditions, salts arising from weathering of the soil tend to accumulate. Such accumulations may ultimately give rise to saline soils. A great many western soils have an appreciable content of soluble salts; in fact, measurement of salt content by means of the Wheatstone bridge is a regular procedure

in western soil surveys. The regions in which this salt content is so great that crop growth is seriously diminished are limited.

Under conditions of low rainfall, vegetation is sparse. The amount of plant material annually returned to the soil is small, and therefore the rate of organic matter accumulation is low. On the other hand, the high summer temperatures and prolonged summer season of the West are conducive to rapid decomposition of organic matter. As Nikiferoff (14, p. 936) points out, these processes leave the desert soils very poor in organic matter. At the eastern boundary of the western soils, we have the chestnut soils. These soils have sufficient rainfall, 15-20 inches, to support moderate grass growth, and they are characterized, therefore, by a fair amount of organic matter. As rainfall diminishes westward, we pass through the chestnut, the brown, the serozem, and the desert soils. The organic matter content of the surface 3 feet of these

TABLE 1

*Average compositions of soils of the arid regions of the United States\**

SOIL	ORGANIC MATTER		NITROGEN		P <sub>2</sub> O <sub>5</sub> (PHOSPHORIC ACID)		K <sub>2</sub> O (POTASH)	
	Per cent	Pounds per acre to a depth of 3 feet	Per cent	Pounds per acre to a depth of 3 feet	Per cent	Pounds per acre to a depth of 3 feet	Per cent	Pounds per acre to a depth of 3 feet
Desert.....	0.3	36,000	.02	2,400	.17	20,400	2.2	264,000
Serozem.....	0.7	84,000	.05	6,000	.18	21,600	2.2	264,000
Brown.....	0.9	108,000	.07	8,400	.17	20,400	2.0	240,000
Chestnut.....	1.1	132,000	.10	12,000	.12	14,400	2.4	288,000

\* Data from *Atlas American Agriculture* and unpublished data Bureau Plant Industry. Supplied by L. T. Alexander.

soils decreases from 132,000 pounds per acre in the chestnut soils to 36,000 pounds in the desert soils, as is shown in table 1.

#### PLANT PROBLEMS ON ARID SOILS

In dry farming, where no irrigation water is added, the greatest limiting factor to plant growth is, in most years, lack of moisture. We know from experience that addition of fertilizer and maintenance of all other plant growth factors at a maximum will help but little in years of low rainfall. This also follows from the generalized yield equation as developed by Mitscherlich, Baule, and Reidemeister (16). The same soils may be irrigated, yet here when the masking effect of moisture as a limiting factor is removed, we can soon discern and study to better advantage the other soil conditions and growth factors and how they affect plant growth.

In addition to its action as a plant growth factor, *per se*, moisture in the soil modifies greatly the rate of bacterial and chemical processes. In this way, water governs the availability of other plant foods such as phosphorus, or the production and accumulation of nitrates.

### *Phosphorus availability*

A recent congressional investigation of the phosphate resources of the nation shows that there are vast deposits of rock phosphate in the States of Idaho, Utah, Montana, and Wyoming. From table 1 we see that the total  $P_2O_5$  content of western soils is high, averaging about 0.16 per cent. Nonetheless, the surface soils in some of these areas may be deficient in phosphorus available to plants. A map published in *Technology on the Farm* (7, p. 118) indicates areas of the United States where the feed grown is deficient in phosphorus for animal feeding purposes. On this map, a large part of the State of Montana is shown as deficient in available phosphorus, and because of soil similarities, other states to the south are believed to be in the same category. This agrees with the general response by plants to phosphate fertilizers in this area.

Western soils, like all other soils, furnish phosphorus to plants from both the organic and the mineral components. The rate at which phosphorus becomes available from the organic fractions depends mainly on whether or not conditions are favorable for bacterial and fungal decomposition of the organic matter.

The amount of phosphorus made available in this way depends on the amount of organic matter present and its rate of decomposition. The higher soil temperatures in the desert increase rate of decomposition, whereas low moisture contents diminish the rate. In the desert regions the net effect is a rather low rate of availability of organic phosphorus.

The mineral part of these soils tends to fix phosphorus. Hibbard (6) lists the factors influencing phosphorus fixation in western soils as follows:

Soil reaction is the most important factor governing phosphate availability, with high pH values decreasing this rate. High soluble and replaceable calcium-ion contents also decrease the availability of phosphorus. The fixing power of soil colloids for phosphorus varies inversely with the silica-sesquioxide ratio  $\left( \frac{SiO_2}{R_2O_3} \right)$ .

Western soils with but few exceptions are alkaline in reaction. Under such conditions, the addition of insoluble phosphorus fertilizers such as raw phosphate rock is almost without effect. This was stressed by a number of state representatives at a phosphate conference held in Ogden, Utah, September, 1940. Addition of soluble phosphorus fertilizers such as superphosphate or ammonium phosphate is beneficial in many instances, provided large quantities are used. Sugar beet, a widely grown western crop, responds readily to soluble phosphorus applications, and over half the acreage undoubtedly receives such fertilization. McGeorge and Breazeale (9) consider that the soluble phosphorus is precipitated in alkaline soils as a very insoluble basic carbonate-apatite. Buehrer (2) in a study of ionization constants of soil phosphates, and particularly the system  $CaHPO_4$ - $CaCO_3$ - $H_2CO_3$ , showed that the equilibrium phosphate concentration is directly proportional to the hydrogen-ion concentration and inversely proportional to the calcium-ion concentration. Since

according to McGeorge (11) the pH of the soils containing calcium carbonate may be as great as 9.4 to 9.7, solubility of native phosphorus is very low. The cost of reducing soil pH values by additions of acid is usually prohibitive, but sulfur shows promise. Sulfur on oxidation forms sulfuric acid.

McGeorge and Frazier (12) have applied sulfur in bands around citrus trees to cause local zones of reduced pH. Under such conditions triple superphosphate was more effective in producing yields, that is, less phosphorus was fixed by the sulfur-treated soil than by the untreated soil. It would seem desirable in calcareous soils such as occur in the Salt River Valley of Arizona, to use local application of phosphorus fertilizers in the root zone. This can be done as band or spot applications with most crops. Coarsely granulated fertilizers would also seem to be desirable for the same reason, that is, the fertilizer is not intimately mixed with the soil and thus reduces the formation of insoluble or difficultly soluble compounds in which the phosphorus is only slightly available to plants. If most of the fertilizer remains out of contact with the soil, the phosphorus within this fertilizer volume is still highly available to the plant roots which penetrate it. More recently the nature of the clay mineral has been shown markedly to affect phosphorus availability. Thus, Stout (18) has shown that kaolinite will fix very large quantities of phosphorus by exchange of OH ions for phosphate ions. Bentonitic clays, on the other hand, do not appear to fix phosphate in this manner.

#### *Chlorosis and minor element deficiencies*

One of the major plant problems on western soils is that of chlorosis. "Chlorosis" is a generic term, of course, but the principal cause for chlorosis in the West appears to be lime in the soil and the problems of malnutrition associated with calcium carbonate and high pH values. In some areas in Idaho, for instance, cottonwood trees have greenish yellow foliage.

Many writers have discussed the relationship between calcium carbonate, high soil pH values, and chlorosis. It was early believed that chlorosis occurred because of an iron deficiency (1). We now know that there are many types of chlorosis. Some occur on acid or neutral soils for lack of essential minor elements such as zinc (3) and manganese (15), but deficiencies of this type are far more common on alkaline soils. In other areas ferrous sulfate sprays are decidedly beneficial. Chlorosis is a major problem in the West. It is present in most of the irrigated areas and is very severe on some crops.

The usual treatment of field crops has been to check the crops for deficiencies and to supply these as spray applications. For tree crops, tree injections, spray applications, and soil applications of the deficient mineral are used.

That the character of the rootstock is also a factor in chlorosis has been shown by Hendrickson (5) for pears. He found *Pyrus communis* to be a better stock under calcareous conditions than *Pyrus serotinus*. On similar soils, Wann (19) found Concord grape scions on Vinifera rootstocks resistant to chlorosis, which is prevalent with other common rootstocks. According

to Baker,<sup>1</sup> of Idaho, prunes on plum rootstocks fared much better on high lime soils than did prunes on peach stocks.

### *Calcium*

McGeorge (10) has suggested that because of the high pH value in some Arizona soils, plants may lack available calcium.

On a number of such high pH soils only a trace of soluble calcium could be found. Additions of sulfur or acid usually improved plant growth, but this may have been due to increased availability of elements other than calcium. Calcium nitrate as a source of nitrogen has been more beneficial than other forms in several areas of the Salt River Valley of Arizona having high soil pH values. In such high pH soils carbon dioxide is low. Additions of organic matter usually improve these soils, and it may be that liberation of appreciable quantities of carbon dioxide reduces the soil pH below the critical limits and renders plant foods, including calcium, available.

### *Organic matter*

It is difficult to maintain an adequate supply of organic matter in western soils. Under conditions of hot summer temperatures and, in irrigated regions, of optimum moisture, organic matter is rapidly oxidized. In these soils a store of organic matter is greatly to be desired, first, as a soil conditioner, especially on soils which are high in clay or are deflocculated by a high content of sodium salts; second, as a source of carbon dioxide to regulate soil reaction and plant food availability; third, as a source of major plant foods such as nitrogen and phosphorus; and fourth, as a concentration medium for some of the micronutrients such as zinc, copper, and molybdenum.

In reclamation practices on saline soils, growth and incorporation of organic matter, usually as a green manure crop, is one of the favored practices. That organic matter is a potent source of nitrogen has long been known, but that organic phosphorus may constitute a significant part of the phosphorus taken up from a soil has been realized only in the last decade.

With loss of organic matter, the fertility of the soil may be seriously impaired. As an example of this, Stephens (17) has tabulated the wheat yields obtained over the past decade on plots in eastern Oregon receiving various organic matter treatments. In this region, rainfall is deficient, and fallowing to conserve moisture is a widespread practice. In spite of better fallowing practice, wheat yields are decreasing where the practices do not return organic matter to the soil. These soils when virgin, contained 0.113 per cent nitrogen in 1912. In 1922, the value was 0.088 per cent, and in 1932, 0.077 per cent. When, however, barnyard manure or mineral nitrogen is applied or legumes are grown, wheat yields are decidedly greater. Under irrigation conditions it is possible to add soluble fertilizers to the water supply. A part of the nitrogen fertilization on the West Coast is now added as ammonia to the ir-

<sup>1</sup> Private communication.

rigation water. The practice tends to counteract increasing salinity because the ammonia is rapidly oxidized to nitrate and no unassimilated cation or anion is added. The use of ammonium sulfate is also widespread on calcareous soils because this fertilizer material is acid forming. The increased acidity renders more calcium soluble and usually improves saline agricultural lands.

### *Soil salinity*

A large proportion of soils in the West contain appreciable quantities of soluble salts. These salts have arisen by soil weathering, and because the rainfall has not been sufficient to leach them away, they remain or are accumulated in the soils of lower elevations, forming our saline or "alkali" soils. Because such soils may be unproductive or difficult to manage, soil surveys regularly make conductivity readings to determine salt contents and indicate these areas on the soil map. The calcium present in the soil solution tends to precipitate as calcium carbonate, leaving sodium, in many instances, as a predominant base. Under these conditions, sodium becomes the principal replaceable base in the soil colloids, the soil becomes impervious, and because leaching is diminished, more salts accumulate. These are the conditions associated with soil salinity.

Crop production on irrigated lands is usually good, but the type of farm management is more exacting than under humid conditions. The 1930 Irrigation Census listed the value of crops on cultivated land of the United States at \$22 an acre and that of crops on irrigated land at \$61 an acre. Irrigated soils of the West are fertile, and with water relations controlled, yields are usually very high.

The West has a relatively small population. The production of vegetables for shipment east is most profitable in the winter months, when only the southern areas have a sufficiently mild climate to allow this. Furthermore, subtropical crops grow only in the south. This leaves the more northern farmer growing irrigated crops such as alfalfa, sugar beets, apples, and potatoes.

The composition and concentration of irrigated waters is of great importance. The water at the source of the western rivers is usually very low in salt content. Water may be removed in the upper parts of the river for irrigation. Evaporation and transpiration increase the concentration of salts in the remaining water and soil. Drainage returns these salts to the river and the river water thus becomes more concentrated. This process is repeated many times in a river such as the Rio Grande. Thus we have the picture shown in figure 2, in which the water near the mountain source contains about 1 equivalent per million of salts, whereas at Fort Quitman, below El Paso, the river contains over 32 equivalents per million.

In the Northwest, waters derived from snow are abundant. A typical analysis of such a snow-fed stream is that of Snake River at Hagerman, Idaho. This is shown in figure 3, together with analyses of irrigation waters of the Rio Grande and of Colorado River, the last two being representative of the rivers



where water is diverted for irrigation at Elephant Butte Dam and at Yuma respectively. For the sake of comparison, the composition of Potomac River at Cumberland, Md. is also shown.

In sandy soils copiously irrigated, the concentration of the soil solution may be but slightly greater than that of the irrigation water. On heavy soils sparingly irrigated and with poor penetration, there may be very little leaching, and as a result the soil solution becomes increasingly concentrated and may be

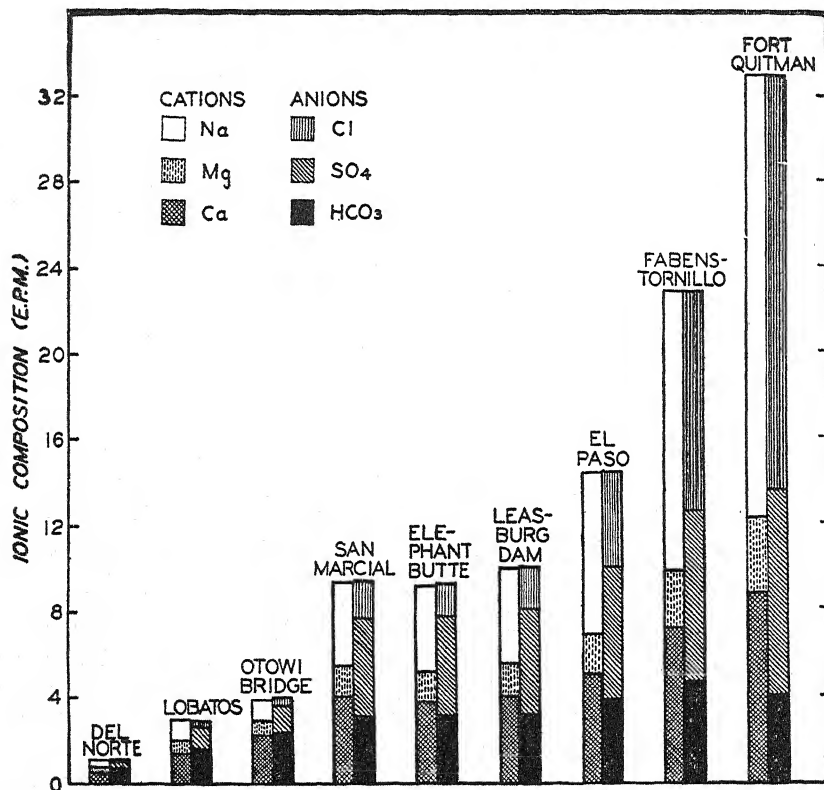


FIG. 2. WEIGHTED MEAN SALT CONTENT OF RIO GRANDE AT DIFFERENT GAGING STATIONS

Data derived from report of Bureau of Plant Industry, pp. 456-461, incl., *Regional Planning, Part VI, Upper Rio Grande, National Resources Committee, 1938*

as much as 150 times as concentrated as the original irrigation water. At concentrations of the soil solution or nutrient solution up to 20 equivalents per million most plants show little decrease in yield. At increasing concentrations, yields fall off much faster. Unpublished work of the U. S. Regional Salinity Laboratory, a cooperative Regional Laboratory between the western states and the U. S. Department of Agriculture, has shown that the reaction of the plant to salt concentrations depends on the total concentration, the kind

of plant, the kind of salts present, and the climate, and we are confident that the soil type will be found to be extremely important.

Almost nothing is known regarding the mechanism of how the more concentrated soil and nutrient solutions reduce plant growth or concerning the effects on the quality of fruit or on the structure of the plants. We do know that alfalfa grown on the more concentrated solutions, for instance, becomes more

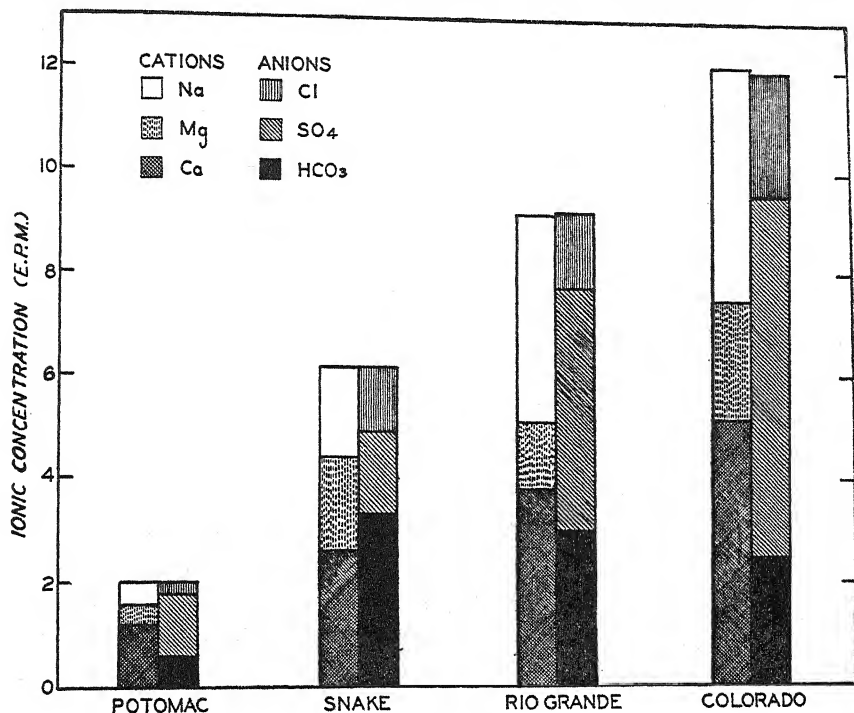


FIG. 3. WEIGHTED MEAN SALT CONTENT OF REPRESENTATIVE RIVERS IN EASTERN AND WESTERN UNITED STATES

Potomac—*U. S. Geol. Survey Water-Supply Paper 236: 93, 1909.*

Snake—at Hagerman, Idaho. *U. S. Geol. Survey Water-Supply Paper 774: 174, sample 2.*

Rio Grande—at Elephant Butte, New Mexico. *Regional Planning, Part VI, Upper Río Grande, p. 458, National Resources Committee, 1938.*

Colorado—at Yuma. *Analyses 1939 by Division of Irrigation Agriculture, Bureau of Plant Industry.*

succulent with thicker leaves, but we do not yet know the effect on the feeding value of the alfalfa.

It is very difficult in many instances to establish a crop on irrigated land that tends to be saline. Thus, lettuce is planted on the shoulders of beds in the Salt River Valley of Arizona, for the evaporation from the bed causes such a high salt concentration at the crown of the bed that if lettuce were planted there germination and early growth would be markedly reduced. By planting

in the shoulder of the bed this is avoided. As rain sometimes washes this surface-accumulated salt containing nitrate into the soil and subsoil of the bed, a rain at this stage is equal to a nitrogen fertilization, according to McGeorge, Wharton, and Frazier (13).

Sugar beets and alfalfa may also require special treatments to reduce soil salt concentrations to a point where a stand can be obtained. The older plants will tolerate more salt than will seedlings.

#### RESEARCH NEEDS

A few of the subjects in the field of soils and plants which need investigation, primarily from the standpoint of western regions, are listed as follows without regard to their relative importance:

Better methods of obtaining the soil solution from heavy soils near the wilting point, and methods of analyzing such solutions. There has been marked progress in meeting this problem at the Regional Salinity Laboratory.

Knowledge of the laws governing moisture flow in unsaturated soils. How rapid is the movement? How do salts affect the moisture properties of soils and colloids? How should tile drains be installed for maximum efficiency?

What differences do we find in rootstocks with reference to salt tolerance? Why is *Prunus Davidiana* as a peach stock so tolerant to saline conditions?

Do saline conditions increase seed setting as in alfalfa? What is the effect on fruit quality, on length of cotton fiber, on carotene content of alfalfa?

How can we manage an alkaline calcareous soil so that fertilizers normally precipitated or rendered inactive under such conditions remain available to plants? This is the problem of phosphorus availability, manganese availability, chlorosis. Will granular acid-reacting fertilizers be more effective than the ones used at present?

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## AVAILABILITY OF IONS IN LIGHT SANDY SOILS AS AFFECTED BY SOIL REACTION

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A discussion on availability of ions in light sandy soils of low natural fertility resolves itself primarily into a consideration of the soil factors influencing the utilization of fertilizer materials applied. Two of the soil factors that determine to a large extent the efficiency of fertilizers are fixation and leaching. Fixation of ions into nonexchangeable and difficultly available forms presents a serious problem in heavier soils, but leaching of soluble fertilizer constituents, especially during periods of excessive rainfall, constitutes an equally important factor governing the utilization of ions in light sandy soils with low exchange capacities. Regardless of how readily available the fertilizer material may be, as determined by its solubility, a large portion of the material will not be utilized if the soil conditions are such as to favor its loss by leaching. It will be shown presently, for example, that magnesium is utilized by citrus more completely from magnesium carbonate than from magnesium sulfate when applied in equivalent amounts to acid sandy soils. Thus solubility is not necessarily a good criterion of availability.

In predicting or evaluating the availability of a given fertilizer material under field conditions, due consideration must be given to soil type and soil conditions. Soil reaction is one of the principal factors influencing fixation and leaching of many fertilizer constituents and therefore plays an important role in governing the availability and utilization of ions in light sandy soils. Likewise the availability of the native supplies of the more insoluble nutrients, as for example, conversion of potassium from nonexchangeable to exchangeable forms, is influenced by soil reaction.

In the discussion of the effect of soil reaction on the availability and utilization of ions, reference will be made primarily to the light sandy grove soils of the Norfolk, Blanton, and Eustis series in Florida. These are perhaps the lightest soils utilized for agriculture in this country. The surface layer, in which most of the fine fibrous root system is found and which is seldom more than 6 inches thick, has an exchange capacity between 2 and 3 m.e. per 100 gm. The exchange capacity of the subsurface soil is usually less than 1.5. The base-exchange capacity of these soils is determined largely by the amount of organic matter present. The exchangeable base content of the surface

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layer is considerably higher than that of the subsoil because of its higher organic matter content and greater exchange capacity. The data listed in table 1 show the average amounts of the various constituents found in 55 grove soils of the Norfolk series (20). Because of the low natural fertility of these soils, large amounts of complete fertilizer are applied annually to supply the necessary plant nutrient elements. A 20-year-old grove will receive annually approximately 3,000 pounds of 4-6-8 fertilizer per acre or its equivalent in addition to the so-called secondary elements such as magnesium, manganese, zinc, and copper. In view of the low exchange capacity and the porous nature of these soils and the large amounts of fertilizer applied, losses by leaching are very great indeed.

The history concerning the use of lime and other basic materials in Florida citrus groves serves to illustrate the importance of proper pH control in soils of this texture. In the period from about 1910 to 1917 large amounts of lime were indiscriminately applied to grove soils. Floyd (8) described the injury

TABLE 1

*Average amounts of various constituents found in 55 Norfolk (grove) soils*

DEPTH	EX- CHANGE CAPAC- ITY*	pH	BASE SATURA- TION	EXCHANGEABLE BASES†						AVAIL- ABLE P‡†	ORGANIC MATTER
				Ca	Mg	K	Mn	Zn	Cu		
<i>inches</i>	<i>m.e.</i>		<i>per cent</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>per cent</i>
0-6	2.72	5.56	57.3	520	39	73	3.2	0.91	0.55	408	1.56
0-18	1.26	5.03	17.8	66	6	26	0.8	0.36	0.26	61	0.70

\* Per 100 gm. soil.

† Per acre (2,000,000 lbs. soil).

‡ P determined by Truog's method.

resulting from excessive applications of limestone as "characterized by a frenching of the foliage, a partial defoliation, the presence of multiple buds . . . a bushy, somewhat rosette-like growth of the terminal branches and a dying back of the branches." These symptoms are now recognized as those commonly associated with zinc and manganese deficiencies (4, 5). Camp and Reuther (3) found that groves on sandy soils with pH values higher than 6 were very commonly affected by zinc deficiency. Following this overliming injury, growers were very reluctant to use any form of lime on grove soils until about 5 years ago. In addition, the increasing use of acid-forming fertilizers without the correction of the resultant soil acidity aggravated the depletion of the supplies of the less abundant elements in soils—magnesium, manganese, zinc, and copper—to the extent that these elements became the limiting factors in production in most groves. The great majority of the growers are now supplying these elements either in the spray form or in the fertilizer and are applying dolomitic limestone and other basic materials to maintain soil reaction between pH 5.5 and 6.

Many points have been advanced in explanation of the beneficial results derived from liming, such as direct nutrient effect of calcium and magnesium, stimulating action upon microbiological activity, improvement in the physical condition of the soil, neutralization of hydrogen ions, and precipitation of toxic amounts of aluminum, manganese, and iron. Not all these need be dwelt upon here. Any improvement in the physical condition of sandy soils following an application of lime is negligible. A number of tests made failed to reveal the presence of excessive amounts of aluminum, iron, and manganese in acid sandy grove soils with pH values as low as 4, probably because of liberal use of phosphatic fertilizers and the relatively high content of readily soluble phosphorus of these soils. Indeed, manganese sulfate is now commonly used on acid sandy soils with beneficial results (23), and iron sulfate is sometimes included in the fertilizer mixtures. The favorable effect of lime and other basic materials on microbiological activity is perhaps a significant factor. Experiments on nitrogen sources at Lake Alfred have shown that liming has a marked influence on the rate of nitrification. Inasmuch as part of the nitrogen is usually supplied in the nitrate form, it is questionable whether the more rapid nitrification would account for the apparently better fertilizer utilization at higher pH values. The remarkable initial responses shown by citrus groves to applications of dolomitic limestone to acid sandy soils are no doubt due to magnesium (9, 21). The experiments now in progress at Lake Alfred, however, in which nitrogen, phosphorus, potash, and the secondary elements are applied with and without lime indicate that other beneficial effects follow an application of any basic material to an acid sandy soil. The basic program, in which the soil reaction is maintained at pH 6, is superior to the acid fertilizer program as measured by the general appearance and vigor of the trees as well as the fruit yields. It would seem, therefore, that the maintenance of proper soil reaction must result in a more efficient utilization of the fertilizer materials applied.

#### DEGREE OF BASE SATURATION IN RELATION TO SOIL REACTION

The percentage of base saturation may be defined as the sum of the exchangeable bases, excluding hydrogen, expressed as per cent of the exchange capacity.

$$\text{Per cent Base Saturation} = \frac{\Sigma B}{\text{Exchange Capacity}} \times 100$$

where  $\Sigma B$  represents the sum of the exchangeable bases, excluding hydrogen, expressed in milliequivalents per 100 gm. of soil. The relation of the degree of base saturation to soil reaction is shown in figure 1, in which the percentages of base saturation are plotted against the respective pH values of a number of light sandy soils of Florida. Since by definition, base saturation may be interpreted as the amount of base per unit quantity of total potential acidity, it is evident that the relationship shown in figure 1 is similar to a curve obtained



by titrating a weak monobasic acid with a strong base. Bradfield (1) has shown that the "apparent dissociation constants" of several acid colloidal clays obtained from the titration curves by means of the following well-known relation,

$$\text{pH} = \text{pK} + \log \frac{\text{salt}}{\text{acid}} \quad (A)$$

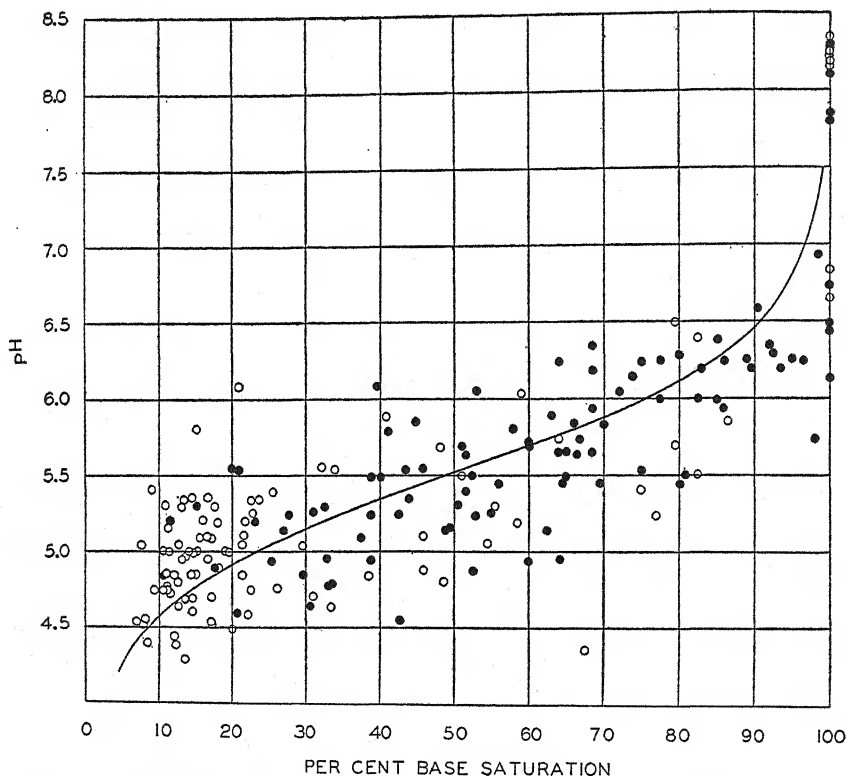


FIG. 1. RELATIONSHIP BETWEEN pH VALUE AND PERCENTAGE BASE SATURATION IN BOTH SURFACE AND SUBSURFACE SOIL SAMPLES

The curve represents a theoretical titration curve of monobasic acid having a pK value of 5.52, which is the "average apparent pK value" found for all of the soil samples. Solid dot, surface soil; open circle, subsoil.

agreed closely with the values obtained by other methods. Applying equation (A) to base exchange data,

$$\text{pH} = \text{pK} + \log \frac{\Sigma B}{\text{Exchange Capacity} - \Sigma B} \quad (B)$$

From the data on the exchange capacity, the sum of the exchangeable bases excluding hydrogen ( $\Sigma B$ ), and the pH value, the average "apparent pK value"

as calculated by means of equation (B) was found to be 5.52 and was used in drawing the theoretical titration curve shown in figure 1. It will be noticed that the base saturation is increased from 25 to 75 per cent by increasing the pH value of a soil from 5 to 6. Regardless of the exchange capacity, the total amount of exchangeable bases (excluding hydrogen) may be expected to increase approximately threefold per unit increase in soil reaction from pH 5 to 6. Hence one of the fundamental reasons for the maintenance of proper soil reaction in light sandy soils with low exchange capacities is to provide adequate supplies of exchangeable calcium and magnesium in order to balance the judicious and economical use of phosphorus and nitrogen. Davis and Brewer (7) have recently pointed out that the available calcium content of many Coastal Plain soils is probably too low for proper assimilation of phosphorus and nitrogen. Careful pH control for this reason is of lesser importance in heavier soils with higher exchange capacities.

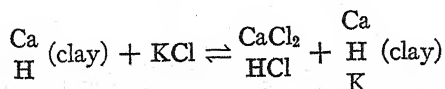
#### LEACHING IN RELATION TO SOIL REACTION

In considering the effect of soil reaction on leaching of plant nutrients in the soil, it seems desirable to differentiate between the native supplies of available nutrients and the added fertilizer constituents. Under acid soil conditions the native supplies of many soil nutrients become depleted more rapidly because of greater dissolution of soil minerals and less soluble compounds. Since the total reserve of plant nutrients is low in light sandy soils of low natural fertility, the present discussion will be concerned primarily with the effect of soil reaction on the fate of some fertilizer materials commonly applied.

Numerous investigations (1, 12) have shown that the adsorbed bases are not equally exchangeable and that the ease with which the various cations are replaced from the soil by other cations from their neutral salts decreases in the order of the usual ionic series;



Lithium is most easily replaced, whereas hydrogen is most difficult to replace and is adsorbed most strongly of all the cations. In addition to the nature of the ion as revealed by its position in the lyotropic series, the concentration of the ion, the degree of dissociation, and the solubility of the end products resulting from the exchange reaction also determine the extent of the exchange. Peech and Bradfield (19) found that the adsorption of potassium from potassium chloride by colloidal clay increased rapidly with the increasing degree of calcium saturation, as shown in figure 2. In the reaction, which may be represented,



the adsorption of potassium was largely due to the replacement of the adsorbed calcium. Though comparatively little hydrogen is exchanged by cations from their neutral salts, it is readily replaced by lime and other basic materials commonly used to correct soil acidity, and the following reaction proceeds to the right with the evolution of carbon dioxide:

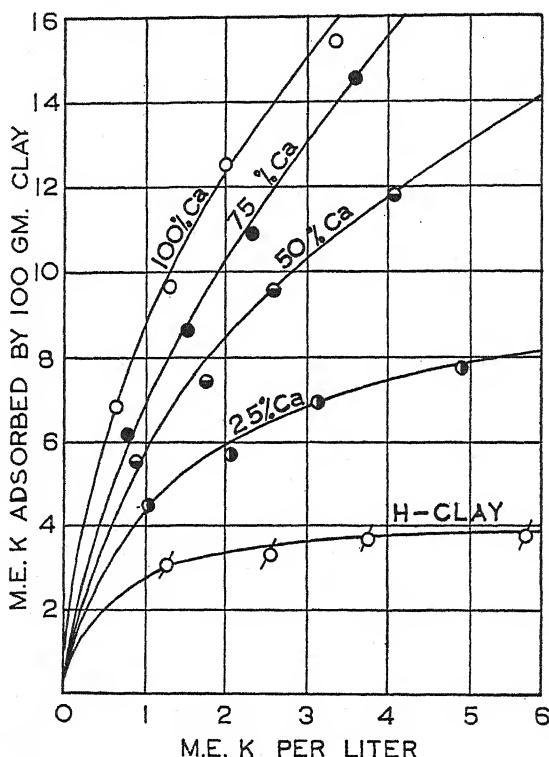
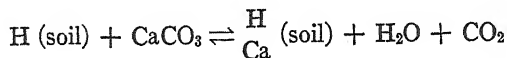


FIG. 2. RELATION OF THE DEGREE OF CALCIUM SATURATION OF COLLOIDAL CLAY TO THE ADSORPTION OF POTASSIUM FROM POTASSIUM CHLORIDE

From the above considerations it would seem logical to expect that application of lime to an acid soil should favor the adsorption and thus reduce leaching of fertilizer materials applied in the form of neutral salts of strong acids such as KCl,  $\text{K}_2\text{SO}_4$ ,  $\text{MgSO}_4$ , and  $(\text{NH}_4)_2\text{SO}_4$ . Unfortunately, the validity of this generalization as applied to the various neutral fertilizer salts has not been sufficiently tested under field conditions, but a few experiments will be cited to illustrate the principle.

In a comparison of different sources of magnesium, varying amounts of magnesium carbonate and magnesium sulfate were applied annually to Norfolk

fine sand with an initial pH value of 5. The analytical results obtained at the end of the third year are listed in table 2 to show the relative effectiveness of the two sources of magnesium in increasing the exchangeable magnesium content of the soil. It will be noted that despite the heavy annual applications of magnesium sulfate, the amount of exchangeable magnesium in the soil was not appreciably increased, indicating that magnesium in this form is subject to rapid loss by leaching in acid sandy soils. On the other hand, magnesium carbonate, especially at higher rates of application, was very effective in building up the exchangeable magnesium content of the soil. Analyses of the foliage from the trees in these plots made by Fudge (10) showed that on the basis of equivalent amounts of magnesium applied, the trees actually utilized magnesium much more efficiently from magnesium carbonate than from the more soluble magnesium sulfate. When magnesium sulfate, however, was used in conjunction with lime on an acid soil or applied after the correction of soil

TABLE 2

*Changes in exchangeable magnesium content and soil reaction induced in Norfolk fine sand following three annual applications of magnesium carbonate and magnesium sulfate*

ANNUAL APPLICATION	MATERIAL APPLIED				
	Magnesium Carbonate (41 per cent MgO)			Magnesium Sulfate (30 per cent MgO)	
	pH	Ca found	Mg found	pH	Mg found
<i>lbs.*</i>		<i>lbs.*</i>	<i>lbs.*</i>		<i>lbs.*</i>
Check	5.15	335	8	4.90	8
100	5.35	485	17	4.85	10
200	5.30	445	22	4.80	11
400	5.45	465	38	4.80	14
800	6.35	665	161	4.85	19

\* Per acre (2,000,000 lbs. soil).

acidity, this loss of magnesium by leaching was greatly reduced. It will be noted in table 2 that the exchangeable calcium content of the soil treated annually with 800 pounds of magnesium carbonate per acre was approximately twice that of the check plot. This increase in exchangeable calcium cannot be attributed to impurities in the high-grade precipitated magnesium carbonate used in the experiment, but is more likely due to the greater efficiency of the soil at higher pH values to adsorb calcium from neutral salts introduced with the superphosphate in the mixed fertilizer. Ordinary superphosphate, containing about 50 per cent calcium sulfate, was used as a source of phosphorus in this experiment. It is apparent from table 2 that calcium applied as superphosphate was subject to loss by leaching in the acid soil as shown by analysis of the soil in the check plot, but the substitution of magnesium for the adsorbed hydrogen in the soil treated with magnesium carbonate favored the adsorption of calcium from this source, magnesium being the cation exchanged.

In order further to demonstrate leaching of cations applied as neutral salts in soils at various pH values, a laboratory experiment was set up with three soils. Soil 1, Norfolk fine sand, was taken from a citrus grove; soil 2 was a hydrogen-saturated soil obtained by leaching soil 1 with hydrochloric acid followed by leaching with water to remove the free acid; soil 3 was virgin Norfolk fine sand. The analyses of the three soils are given in table 3. Known amounts of standard solutions of magnesium chloride, potassium chloride, and manganese sulfate were added to a series of 50-gm. samples which had been previously adjusted to different pH values with calcium hydroxide. The soils were then brought to 50 per cent moisture content and allowed to stand for 48 hours, after which time sufficient water was added to make a 1:2 soil-water suspension. The suspensions were then stirred and filtered. Calcium, magnesium, potassium, and manganese were determined in aliquots of

TABLE 3  
*Chemical composition of soils used in studies on leaching and fixation*

SOIL NUM- BER	DESCRIPTION	EX- CHANGE CAPAC- ITY*	pH	EXCHANGEABLE BASES†				AVAIL- ABLE P†‡	WATER- SOLU- BLE P†§
				Ca	Mg	K	Mn		
		m.e.		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1	Norfolk fine sand, grove soil	2.04	5.10	580	10	23	2.0	545	19
2	Same as soil 1, hydrogen-saturated	2.00	3.10	13	2	12	0.8	20	16
3	Norfolk fine sand, virgin soil	2.08	5.30	155	29	20	3.5	20	4

\* Per 100 gm. soil.

† Per acre (2,000,000 lbs. soil).

‡ P determined by Truog's method.

§ P soluble in 1:5 soil-water extract.

the extracts, and the analytical results, listed in table 4, show the amounts of the various constituents applied as well as the amounts found leached at different pH values. It will be noted that increasing the pH of the three soils resulted in considerable reduction of the amounts of magnesium and manganese leached but had very little effect on the leaching of potassium. Inasmuch as the base-exchange property of these soils is derived largely from the organic matter (20), it is possible that potassium forms soluble organic complexes at higher pH values and leaches in this form. This may explain why leaching of potassium from neutral salts was not appreciably affected by soil reaction in these soils. The results of this experiment substantiate those obtained under field conditions and would support the conclusion that potassium, in contrast to calcium, magnesium, and manganese, applied in the form of neutral salts is subject to rapid leaching in light sandy soils regardless of soil reaction. It is probable that the diminution in the amounts of magnesium and manganese

leached at higher pH values may be attributed partly to the formation of insoluble compounds, but it will be noted that a rapid reduction in leaching of these cations with increase in pH occurred in the lower pH range. Furthermore, soil 2, from which virtually all of the readily soluble phosphorus was removed, as shown in table 3, gave results similar to those of the original soil (No. 1). It would appear, therefore, that the reduction in leaching of magnesium and manganese noted above was primarily due to the greater efficiency of the exchange complex to adsorb cations from neutral salts at higher pH values.

TABLE 4

*Effect of soil reaction upon leaching of magnesium, potassium, and manganese, applied as neutral salts, at different pH values*

SOIL NUM- BER	pH	APPLIED*†				LEACHED*‡			
		Ca	Mg	K	Mn	Ca	Mg	K	Mn
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1	5.0	40	88	77	22	210	62	72	7.5
	6.0	280	88	77	22	226	54	69	4.5
	7.0	600	88	77	22	275	44	69	2.3
	8.0	1040	88	77	22	325	38	73	0.8
2	3.1	0	88	77	22	16	66	72	13.0
	4.0	535	88	77	22	73	54	66	8.0
	5.0	1000	88	77	22	130	51	65	4.7
	6.0	1500	88	77	22	146	37	63	2.5
	7.0	1820	88	77	22	195	35	65	1.8
	8.0	2480	88	77	22	260	33	65	0.8
3	5.2	0	88	77	22	32	63	67	10.0
	6.0	320	88	77	22	57	48	64	4.9
	7.0	880	88	77	22	120	37	64	2.3
	8.0	1560	88	77	22	250	33	64	1.0

\* Per acre (2,000,000 lbs. soil).

† Calcium applied as hydroxide; magnesium and potassium as chlorides; manganese as sulfate.

‡ Found in 1:2 soil-water extract.

#### FIXATION IN RELATION TO SOIL REACTION

It has been shown that the addition of lime to correct excessive soil acidity reduces losses by leaching and thus helps to conserve the more valuable fertilizer constituents applied in the form of neutral salts. Raising the pH value, however, by the indiscriminate use of lime to the point favorable to fixation of ions into nonexchangeable or more insoluble forms may offset any benefits derived from liming. The order in which the deficiencies of different elements subject to fixation at higher pH values are induced by overliming will vary in accordance with the requirements of different crops. Naftel (18) and others

(15) have recently shown that overliming injury to a number of annual crops can be overcome by the application of boron. That soluble manganese salts are readily oxidized and precipitated as manganese dioxide in alkaline soils is well known. Some of the calcareous soils in Florida that had received over 1,000 pounds of manganese sulfate per acre showed upon examination less than one pound of exchangeable manganese in the surface 6 inches of soil (4). Camp and Reuther (3) and Mowry and Camp (16) found that zinc deficiency in both citrus and tung oil trees can be readily induced by liming above pH 6. This is in agreement with the work of Lott (14), who reported that the toxicity of heavy applications of zinc oxide could be inhibited by the addition of lime to raise the pH of the soil to 6. Recent investigations of Hibbard (11) have also shown that zinc is not readily replaced by cations of neutral salt solutions and that only acid solvents extract appreciable amounts of zinc from soils. The results of some of the preliminary experiments reported below would tend to substantiate the field observations—that both zinc and copper are fixed even in light sandy soils at comparatively low pH values.

The soils used in this study were those listed in table 3. Fifty-gram samples of each of the three soils were adjusted to different pH values with calcium hydroxide. After the establishment of equilibrium, as shown by pH measurements, known amounts of standard solutions of zinc sulfate and copper sulfate were added, and the soils were brought to 50 per cent moisture content. After they stood for 48 hours, sufficient water and 2 *N* sodium chloride solution were added to make the final volume of the solution 100 ml. and 1 *N* with respect to sodium chloride. The suspensions were stirred thoroughly, siphoned after standing for 15 minutes, and centrifuged. Zinc and copper were then determined in the supernatant liquid by the methods previously described (20). The amounts of zinc and copper recovered by single extraction with 1 *N* sodium chloride solution from the three soils were found to decrease rapidly with the increase in pH of the soil, as shown in figures 3 and 4. The recovery of copper was considerably lower than the recovery of zinc at any given pH, indicating that copper is fixed the more strongly of the two cations. Whereas all the zinc added to soil 2 was recovered at pH 3, only 40 per cent of the copper was extracted from the same soil at this pH value. Another noteworthy observation is that the three soils behaved alike toward the fixation of zinc and gave virtually the same results, as shown in figure 3. Judging by the variation of the phosphorus content of the three soils, it is not likely that the fixation of zinc can be attributed to phosphorus, as has been suggested by West (25). It should be also pointed out that the results reported here are somewhat at variance with those published by Jones, Gall, and Barnette (13), who found that zinc applied as zinc sulfate in amounts of less than 300 pounds of zinc per acre to Norfolk sand was fixed in the replaceable form. The amounts of zinc and copper fixed, as measured by single extraction with 100 ml. of 1 *N* sodium chloride solution, when varying amounts of standard solutions of zinc sulfate and copper sulfate were added to 50-gm. samples of soil 1 adjusted to pH 6 and the suspensions allowed to stand for 48 hours, are shown in figure 5.

It is apparent that copper is fixed more strongly than zinc, despite the fact that both are precipitated as hydroxides (or basic salts) at this pH value (2)

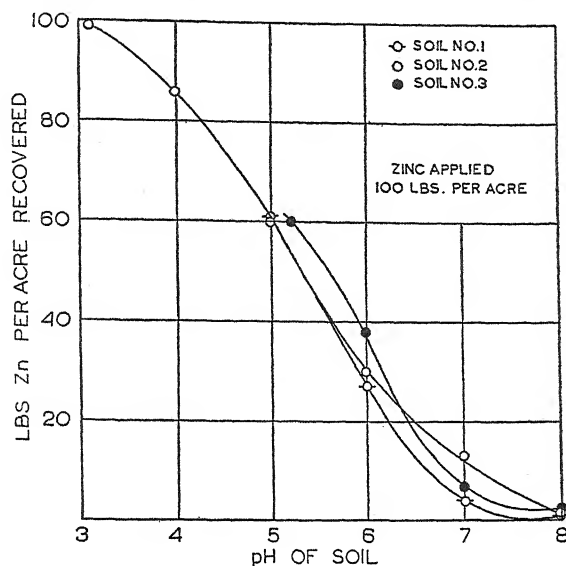


FIG. 3. RECOVERY OF ZINC APPLIED TO SOILS AT DIFFERENT pH VALUES BY SINGLE EXTRACTION WITH 1 N SODIUM CHLORIDE SOLUTION

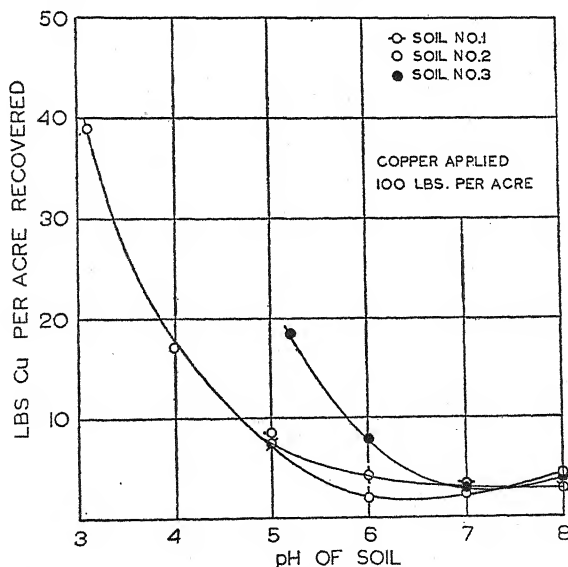


FIG. 4. RECOVERY OF COPPER APPLIED TO SOILS AT DIFFERENT pH VALUES BY SINGLE EXTRACTION WITH 1 N SODIUM CHLORIDE SOLUTION

and that the solubilities of the two hydroxides are of the same order of magnitude (22). Since copper forms more insoluble combinations than zinc in the



soil, it is difficult to reconcile frequent failures of heavy soil applications of zinc sulfate at the rate of 10 pounds per tree to correct zinc deficiency (3, 6),

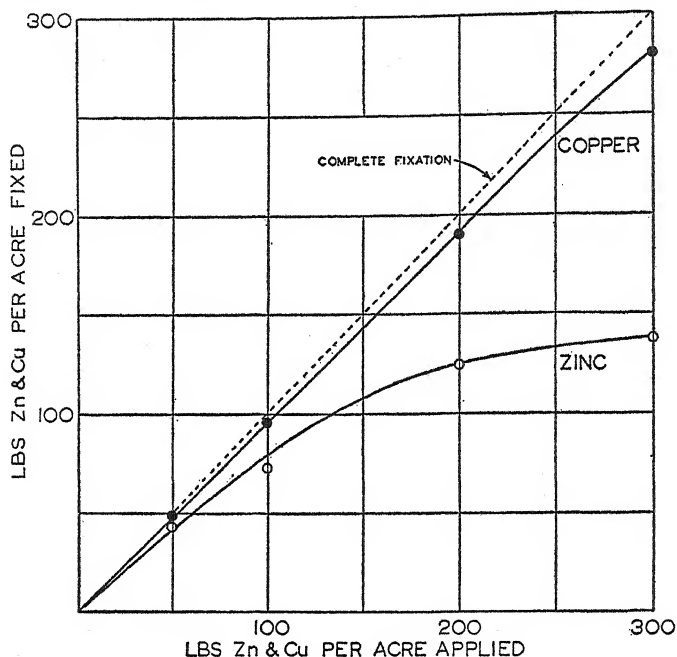


FIG. 5. FIXATION OF ZINC AND COPPER BY NORFOLK FINE SAND AT pH 6 AS MEASURED BY SINGLE EXTRACTION WITH 1 N SODIUM CHLORIDE SOLUTION

TABLE 5

*Changes in available phosphorus content induced in Norfolk fine sand following three annual applications of high-calcium limestone*

ANNUAL APPLICATION*	pH	AVAILABLE P*†
<i>lbs.</i>		<i>lbs.</i>
Check	4.85	225
200	5.30	255
400	5.50	300
800	6.20	352
1600	6.85	335
3200	7.00	400
6400	7.15	315

\* Per acre (2,000,000 lbs. soil).

† P determined by Truog's method.

whereas much smaller applications of copper seldom fail to control symptoms of copper deficiency.

Soil reaction also has a marked influence on the availability of phosphorus.

Phosphorus is fixed by iron and aluminum in acid soils, whereas at higher pH values it reverts to tricalcium phosphate, the optimum pH being around 6.0. Typical results showing the effect of increasing applications of lime on the amount of readily soluble phosphorus as determined by Truog's method (24) are listed in table 5. This is in accord with findings of other investigators (17).

#### SUMMARY

Two of the important factors that govern the availability and consequently the utilization of ions in light sandy soils under field conditions are leaching and fixation, which in turn are controlled by soil reaction. If the amount of an element utilized by the plant may be taken as a measure of availability of that particular element, then solubility is not necessarily a good criterion of availability.

The correction of excessive acidity of light sandy soils by liming not only assures adequate supplies of available calcium and magnesium but also reduces leaching of cations, applied as soluble neutral salts, by favoring their adsorption into exchangeable form, and thus tends to conserve the more valuable fertilizer constituents.

The indiscriminate use of lime, however, to raise the pH value of the soil to the point favorable to fixation of ions into nonexchangeable and nonavailable forms may offset any benefits derived from liming. Among the elements that are rendered unavailable by overliming, zinc and copper are fixed at comparatively low pH values.

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# SOIL ORGANIC MATTER AND ION AVAILABILITY FOR PLANTS<sup>1</sup>

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Recent progress in understanding the mobility of cations around the colloidal mineral part of the soil is sufficiently satisfactory to prompt inquiry about ion mobility in relation to the organic matter fraction of the soil. Even though anion behavior, like that of phosphorus, is still a secret, yet the lack of a clear concept of all mineral phases need not deter us from giving some, more or less speculative, attention to ion mobility centered about the organic matter. When this small part of the soil renders such magnitudinous service in plant production, it may well have attention with the hope that an understanding of its behavior will elucidate, in some additional measure, the complex phenomena of plant nutrition.

## DIFFERENT PHASES OF ORGANIC MATTER BEHAVIOR AND ION MOBILITY

The scope of organic matter behavior may well be outlined, at the outset, by reminding ourselves that soil organic matter may be both positive and negative in its effects. It may contribute positively in mobilizing ions for better or more rapid delivery to the plants. Contrariwise, it may serve to demobilize, or fix, and remove them from plant access. Effects in these opposite directions may be exercised simply in a physical way by the ion adsorption on the colloidal organic matter. Ions may be thus removed from solution and held so firmly adsorbed as to become a negative factor in plant growth. On the other hand, the ions may be adsorbed at so much higher degrees of saturation as to be delivered more readily for plant service through the simple processes of ionic exchange.

The breakdown of the organic matter through the decay process may serve in a positive chemical way by contributing nutrient cations and anions to the plants, just as any burning process delivers ash. A contrary chemical effect may result, however, when the composition of the decaying compounds fails to serve as a well-balanced bacterial ration and compels the microorganisms to withdraw ions from the soil solution or from the absorption complex and put into immobile form as insoluble microbial complexes many otherwise mobile ions. Because bacteria can operate at lower concentrations than more highly developed plants, this ion demobilization represents a microbial

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competition with plants. These aspects of ion mobility and ion immobility in both physical and chemical ways must be appreciated if the role of organic matter in connection with ion delivery to the growing crop is to be clearly understood.

#### DECLINING SOIL FERTILITY MEANS DECLINING AMOUNT AND VALUE OF SOIL ORGANIC MATTER

Farm manure has been considered the "foolproof" fertilizer of the past. It will not necessarily be so considered in the future if it is produced on, and returned to, soils excessively tilled and depleted of their store of mobile mineral nutrient ions. Manure, which has been valued, in the main, for its nitrogen content must be valued also for the phosphorus, the potassium, the calcium, the magnesium, and all the other contributions it makes as it gives up its mineral constituents of the past plant generations to create those of the future. The successively diminished store of ions offered by the soil is lowering the value of the manure as it is produced from feeds and roughages grown on such soils. By merely going back to the point whence it came, organic matter can do little to uplift the productivity. Manure is not saturated with active nutrient ions purely because it represents vegetative matter that went through the animal's digestive mill. On soils of declining fertility each cycle of organic matter through decay and plant growth represents vegetation of type and composition still lower in fertility level. Manures produced therefrom must reflect the advancing fertility deficiencies as they represent woody organic matter rather than organic matter loaded with nitrogen, minerals, and other nutrients contributed by the soil.

The growth of fungi on manure in the form of commercial mushroom production, according to some preliminary studies,<sup>2</sup> is suggesting that declining soil fertility with its increasing soil acidity, or rather its decreasing exchangeable calcium, is reflecting itself in terms of lowered mushroom-producing capacity from much of the manure being produced at present. Perhaps this is traceable to the lower mineral effectiveness in the straws of the bedding, in the feces, and in the urine of the manure in the mushroom compost. Perhaps it may be located also in the fertility-deficient casing soil as it fails to compensate for these defects in the manure.

Organic matter merely coursing regularly through its cycle of vegetative growth, decay, and reincorporation into a new crop of organic matter as a natural process represents ion mobility, but is not a means of increasing the mass of ionic nutrients in the cycle. On the contrary, it is nature's slow method of holding this mass from slipping downward rapidly. In the humid regions, organic matter is not an elevator of production purely because it is of organic nature. Rather the improved fertility in terms of more calcium, more magnesium, more phosphorus, more potassium, and other similar items is the

<sup>2</sup> Schroeder, R. A. Unpublished report. Department of Horticulture, Agricultural Experiment Station, Columbia, Missouri.

means of providing a greater mass of organic matter. This consists also of improved chemical composition and is set for more rapid destruction through its more effective service in microbial nutrition. Consequently, the higher level of soil fertility rotates faster in these larger masses for better plant production. Organic matter in the soil is the effect and not the cause of the mobile ions that represent the fertility supply of the soil.

#### ORGANIC MATTER BEHAVIOR FITS INTO THE COLLOIDAL CLAY CONCEPT

Even though the complete separation of the partly decayed organic fraction—the so-called humus—from the mineral part of the soil has been impossible; nevertheless, the properties of organic matter have been worked out well enough to establish the fact that the bulk of the soil organic matter is colloidal in nature and behavior. Its origin from different materials, in different localities, and under different climatic conditions may well inject variations into its carbon-nitrogen ratio, its ash content, and its general chemical composition. Nevertheless, with lignin and other relatively stable compounds making up a large share of the colloid, the composition of this soil fraction is constant enough to warrant generalization of its behavior into some broader principles.

We now know that chemical reaction is possible between the purely organic and the purely mineral colloidal parts of the soil (7). The organic colloid and the soil mineral colloid are similar in behavior and properties. The acid organic matter colloid from peat acts toward hydrogen replacement from its complex by other ions according to the order of their increasing valency—a behavior in close agreement with that of colloidal aluminosilicate, or clay (5). The humus colloid is, however, more highly hydrated. Its union with calcium gives a floccule of about three times the volume of that produced by the union of calcium with the Putnam-clay colloid. Hydrogen and calcium have many similar effects, whether combined with the colloid that is humus or with that which is a mineral clay. The acid organic matter, or hydrogen humus, however, is the more stable in water, the calcium humus being about four times as easily resuspended after drying or flocculation.

#### HYDROGEN HUMUS MAY DECOMPOSE MINERALS

That the colloidal hydrogen humus may mobilize cations out of the mineral crystal lattice and into its own ionic atmosphere from which these cations may enter the plants more effectively, has recently been established by Graham.<sup>3</sup> Pure, freshly pulverized, and acid-washed anorthite particles of silt size, when mixed with electro dialyzed hydrogen humus, gave up calcium to the organic colloid at rates about three times as great as to the acid, or hydrogen, clay. Successful tests with the anorthite-treated clay for plant nourishment by the calcium so mobilized (6) suggest that the colloidal acid humus may be serving similarly. If this is a fact, then organic matter is not only mobilizing the stock

<sup>3</sup> Graham, E. R. Unpublished report. Department of Soils, Agricultural Experiment Station, Columbia, Missouri.

of ions constituting its own complex, or even not only those caught by adsorption from solution as they were passing by, but it is also apparently quarrying some cations from rock fragments within the soil to mobilize these also.

These fundamental properties of organic matter as a colloid are helpful in understanding ion mobility and possible offerings of nutrients to plants through the many activities of this small fraction of the soil. Ion adsorption and ion exchange as physical or chemical processes by the organic matter fit into the same categories as those by the clay colloid, save that the humus fraction has from two to six times more exchange capacity per unit weight. It is also significant that the ash content of the humus—possibly a reflection of the soil fertility that produced the original humus-forming plants—bears some relation to the exchange capacity. That some variations in the physical and chemical properties of the organic colloid should occur ought not to be seriously disturbing, when the organic matter itself is only an ephemeral compound in the presence of the soil microorganisms using its carbon as an energy source. Such variations are so small that nutrient cation behavior toward organic colloids calls for little revision of the views regarding nutrient cation mobility from or to the colloidal clay fraction which may well serve as the behavior pattern. When anion activities toward both organic and inorganic colloids are equally well cataloged, then plant nutrition will be much better understood.

#### ION MOBILITY AND ORGANIC MATTER IN SOIL DEVELOPMENT

Organic matter is a factor in moving ions about in the soil profile during the processes of soil development. In the process of podzolization under forest vegetation, the leaf litter provides a hydrogen organic colloid which moves downward through the surface horizon of the soil beneath it. The question may well be raised whether the podzolic surface horizon is depleted of its bases wholly because of acidic action of the organic matter traversing it, or whether the soil was not already highly deficient in the bases, particularly in calcium, before the forest vegetation was established there. Since trees live largely by rotating their mineral capital as they drop their leaves to return the minerals to the soil for repeated use, may not the organic matter be a means of holding minerals against leaching and thus of retarding soil development, rather than an agency in leaching out these nutrients in what is commonly credited as the main role of organic matter in the soil development performance known as podzolization?

Though leaves represent rotating mineral capital, yet they are certainly a poor bacterial ration with reference to nitrogen. Their destruction is not rushed to completion, with carbon dioxide and water as end products. Instead, intermediate complex humus compounds result. When organic matter is rich in protein and also rich in the minerals required in larger quantities by protein-producing vegetation, it decomposes so rapidly and so completely that little humus results. Leaf humus is deficient in the growth-inducing elements; namely, nitrogen, phosphorus, calcium, and others, but still is rich in energy-

providing carbon. If it moves down through the soil profile without suffering bacterial destruction, then it seems safe to conclude that it remains unattacked by soil microorganisms, because neither it as a compound, nor the respective soil horizons met enroute, contain the essential nutrients to make microbial use of it possible. As such energy-supplying organic matter goes downward toward clays of higher calcium saturation, it eventually arrives at that degree of offerings of calcium (and probably of other cations) where, supplemented by the soil, it becomes a balanced bacterial ration (4). Under standing water to give anaerobiosis, the gray layer or the glei horizon of podzolic soils develops. There iron is reduced to the ferrous condition as it contributes oxygen for organic matter combustion. The iron becomes soluble and is moved upward or downward as a consequence of the presence of organic matter rather than because of high degree of soil acidity.

In the development of chernozem soils, the organic matter and calcium are both commonly associated through the significance ascribed to the calcium in the preservation of the organic matter. It now seems doubtful whether it is correct to believe that chernozem soils have retained large amounts of organic matter because this was preserved chemically by the calcium, when, in reality, the mobility of the organic matter in combination with calcium is really increased. Calcium is an agency to encourage complete combustion of organic matter (1). The ubiquitous deficiency of calcium points out that it would be more nearly correct to believe that organic matter is accumulating because mineral nutrient deficiencies for the microbes keep it from decomposing. Then too, since calcium humate is a much less stable compound in water than is hydrogen humate, such increased mobility and wider distribution of the dark color through the profile of calcium-laden horizons of open structure are additional encouragement for bacterial destruction rather than for preservation.

Under such conditions the idea of preservation because of the presence of calcium is untenable. A more plausible explanation of the high organic matter content of chernozems is that the liberal supply of calcium and other bases induces more nitrogen fixation, both symbiotic and nonsymbiotic, to produce and to hold more carbon and to increase the organic matter content in spite of the increased rate of its destruction by microorganisms in such a favorable medium. Chernozems represent soil development to the maximum of ion mobility, and of ion-organic matter complexes, all at high levels and at high rates of seasonal turnover. In such a concept of soil development with the help of organic matter, may be the key to high soil productivity under our management.

#### ORGANIC MATTER PRODUCTION AND ION MOBILITY THROUGH PLANT GROWTH

The degree to which a soil has developed, or the extent to which the original rock minerals have moved toward true solution and toward their final resting place in the sea, determines in no small way the ion mobility in any soil. As the organic matter mobilizes or demobilizes various ions, it may be an arresting



or a hastening force to this process. It is in this respect that organic matter—itsself of plant origin—plays no small role in plant nutrition. Some recent studies offer interesting suggestions regarding organic matter as it modifies the mobility of calcium, nitrogen, and hydrogen. To date, the calcium and the hydrogen have stood out among the cations because of their particular properties and because of their presence in magnitudes measurable by delicate laboratory instruments or by particular plant behaviors. Perhaps other nutrient ions will be brought into the picture when sufficiently accurate methods of following their behavior become available.

#### MORE ORGANIC MATTER MAY MEAN MORE EXCHANGEABLE CALCIUM AND MORE EXCHANGE CAPACITY

Studies<sup>4</sup> of the successive 1-inch horizons in the Shelby profile at the Soil Conservation Experiment Station, Bethany, Missouri, show that in a bluegrass sod experiencing no soil treatment and no erosion, the exchangeable calcium was related to the organic matter content. The highest organic matter content (5.71 per cent) had associated with it the highest amount of exchangeable calcium (14.60 m.e.), when for the entire seven horizons (0-7 inches) the figures were, as a mean, 3.73 per cent of organic matter and 11.75 m.e. of exchangeable calcium. This is the situation for the organic matter contributed by the crop of bluegrass, which is not commonly considered a calcophile.

The organic matter accumulation from the bluegrass increased not only the exchangeable calcium, but also the exchangeable magnesium. With successive years in bluegrass sod, plot 8 at Bethany, Missouri, with its increasing organic matter content from 1931 to 1937 showed a progressive increase in base saturation from 77 per cent to 91 per cent. Here, then, the process of soil calcification, or an increasing degree of calcium saturation, was going on under the environmental conditions of north Missouri and while the bluegrass crop was being produced but not removed. Leaching was not an impossibility and may have been occurring, but carbonates were not moved to the lower levels. There was a total deficiency of 22 inches in precipitation during the 7-year period, though the annual precipitations were never lower than 21.8 inches in 1937 and the annual mean for the period was 34.44 inches.

Thus this careful plot study points to organic matter production by grass as a means of leaving within the soil an increased mass of a colloidal residue that is organic in nature, that may be highly saturated by calcium, and that will deliver this nutrient much more rapidly in seasonally timed rates when the sod is broken out and the land put into a tilled crop. This suggests that sod crops as soil fertility restorers may have benefits that are not limited to nitrogen: they may be mobilizing the mineral phases of the soil for their own benefits as well. Here were an increased exchange capacity and an increased degree of calcium saturation, because of the increased organic matter provided through the growth of a sod crop and its period of soil fertility rejuvenation.

<sup>4</sup>Whitt, D. N., and Swanson, C. L. Unpublished report. Missouri Soil Conservation Experiment Station, Bethany, Missouri.

## CALCIFICATION OF ORGANIC MATTER SEEMINGLY ENCOURAGES AZOFICATION

In this bluegrass sod under study, where the base saturation increased from 77 to 91 per cent, of which 90 per cent was calcium, total nitrogen increased from 0.173 to 0.185 for the years 1931 to 1933 and from 0.190 to 0.205 for 1935 to 1937 respectively. This improvement in the nitrogen content of the soil took place while the hydrogen-ion concentration, supposedly a significant factor for azotobacter, registered pH 6.2 for most of the time, though in the first year, 1931 it stood at 5.6. While this change in pH occurred, the calcium saturation increased 18 per cent. It is interesting to note, in passing, from some other studies (3) with bluegrass and redtop, that complete calcium saturation of the soil made the delivery of nitrogen from the soil to the crop most effective. Thus we are inclined to believe that this nitrogen increase at Bethany under bluegrass sod and the increased calcium saturation are not completely separate performances, even if we are not ready to grant that they are causally connected. Nevertheless, with the increase in the organic matter have come an increase in the mobile supply of nutrient ions and a demobilization of the hydrogen ion, their commonly reciprocal ion on the colloidal exchange complex.

## SIGNIFICANCE OF pH MAY BE DWINDLING

Here, as has been previously shown (2), the ions are more efficient with their higher degrees of saturation. Increased mobility of the nutrient ions results not only in increased plant growth above the soil, but also in increased microbial growth and activity within the soil. With more microbial activity, there is a speedier cycle of rotation from soil to crops and back to soil to bring into the cycle other elements not previously involved. That such may have occurred under the bluegrass sod is suggested by the fact that in another plot with a regular crop rotation receiving no treatment and having no soil erosion during these same years, the organic matter content remained constant, when the plow depth was also kept constant to prevent clay incorporation from the subsurface. In those plots showing decreases in organic matter, exchangeable calcium decreased. The amount exchangeable varied with the organic matter while the pH was constant. Here is another indication that pH can scarcely be causally related to crop production if the pH remains constant while the exchangeable calcium and the associated organic matter, both large factors in plant growth, fluctuate so widely.

## OTHER POSSIBLE PHASES OF ION MOBILIZATION BY ORGANIC MATTER

If organic matter plays a role in soil development through the mobilization of ions, which may be viewed as a simple chemical process extending over ages of time, and if its role in ion mobilization for plant nutrition in annual cycles suggests that this latter role supports the soil development processes, then, perhaps, other activities of organic matter will gradually add themselves to complete the picture of the organic matter performances in the soil. It may be our misfortune that the understanding and appreciation of the significance

of organic matter as a mobilizer of nutrient ions will come too late. By the time we fully appreciate the organic matter heritage in our glacial soils as the source of fertility delivered to our crops at rates commensurate with their seasonal demand for profitable production, then the supply will already be so nearly exhausted as to jeopardize the farming business.

We need to stimulate further thinking about the organic matter as a mobilizer of ions when other observations, the exact significance of which is unverified, are indicating that organic matter is playing such a role. Has it been a mere whim of the fertilizer user of the South to insist that a share of his applied fertilizer nitrogen be in the organic form? Is the struggle to find a proper organic-inorganic ratio in fertilizers (8) for southern crops without physiological foundation? Might not the recent attention to the "acidity" of purely mineral fertilizers suggest a case of calcium deficiency that shows up quickly when not covered by organic matter performances within which apparently the calcium supply is automatically increased with the increasing stock of organic matter? Does our recent attention to the many so-called "growth-substances," "accessories," "biotin," and similar growth-stimulating compounds of known chemical structure, suggest that their shortage is manifesting itself because the diminishing organic matter in the soil brings it into prominence? As the knowledge about the chemical structure of these becomes effective for plant growth improvement, it will set us thinking about the kind and composition of organic matter we put into the soil to mobilize nutrient ions. When carbonaceous vegetation with its wide silica-calcium ratio and low content of other soil-derived nutrients is recognized as a *demobilizer* of ions for plants, while leguminous vegetation with a narrow silica-calcium ratio and high content of elements other than silica from the soil is known as a *mobilizer* of such ions, then organic matter will become a widely used tool for better crop production and for more effective soil and fertility conservation. In the organic matter of the soil is the means of managing the fertility of the soil most wisely for its service to the future.

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